AVOCADO MEAL: A NOVEL DIETARY FIBER SOURCE IN FELINE AND CANINE DIETS

BY

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THESIS

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ABSTRACT

With the increasing competition between the human food and the pet food industries for ingredient procurement and availability, the pet food industry is looking for new ingredients that do not compete directly with the human food supply chain. Many by-products from the human food processing industry are under-utilized or destroyed and may be suitable ingredients for the pet food industry. Avocado meal, the ground and dried defatted pulp, seed, and skin after avocado oil processing, has not been used in feline and canine diets, but limited research in rats, sheep, and broiler chickens has shown that avocado meal may be an adequate fiber source.

The overall objective of this research was to assess the use of avocado meal as a novel dietary fiber source for feline and canine extruded diets in terms of processing, extrusion characteristics, and nutritional adequacy. Three diets containing either avocado meal (**AMD**), beet pulp (**BPD**), or cellulose (**CD**) as the dietary fiber source were formulated to meet the AAFCO (2016) nutrient requirements for adult cats and dogs and processed using a single screw extruder. Samples of each diet were taken at each processing stage (raw avocado meal, raw dry ingredient mixes, preconditioner, extruder, dryer, first coating, and second coating). Diets were fed to 8 neutered male cats for 21 days (**d**) and 9 intact female Beagles for 14 d. Periods consisted of 17 d or 10 d of diet adaptation, respectively, with 4 d of total fecal and urine collection. One fresh fecal sample was collected per animal per treatment within 15 minutes of defecation. The avocado meal ingredient, diets (including processing stages), feces, and urine were analyzed for macronutrient concentrations and apparent total tract digestibility (**ATTD**). Fresh fecal samples were analyzed for phenols, indoles, ammonia, short-chain fatty acids [**SCFA** (acetate, propionate, and butyrate)], and branched-chain fatty acids [**BCFA** (isobutyrate,

isovalerate, and valerate)]. Extrudate samples for all three diets from the extruder (**E**), dryer (**D**), and first coater (**CO**) were analyzed for expansion and texture changes.

Our first goal was to characterize the chemical composition of avocado meal and compare the processing of AMD to BPD and CD. The avocado meal ingredient contained moderately low levels of acid-hydrolyzed fat (**AHF**) (9.1%) and crude protein (**CP**) (11.5%) with higher levels of total dietary fiber (**TDF**) (37.4%) [values expressed on a dry matter basis (**DM basis**)]. Soluble dietary fiber (**SDF**) and insoluble dietary fiber (**IDF**) were 27.0% and 10.4%, respectively. We expected chemical composition of the diets to be unaffected by processing, which was observed except for DM, AHF, and fiber fractions. Dry matter decreased with the addition of water and steam at the preconditioner and extruder and increased at the dryer when moisture was removed. Acid-hydrolyzed fat increased at the preconditioner for BPD and CD and at the coater for all three diets due to the addition of choice white grease. In general, TDF and IDF concentrations decreased after extrusion and were diluted with the addition of fat at the coater. Extrudates of AMD and BPD tended to have greater expansion and lower hardness compared to CD.

Our second goal was to assess avocado meal as a novel dietary fiber for feline diets. In most cases, AMD performed similarly to BPD in terms of macronutrient apparent total tract digestibility and fecal fermentative end-product concentrations. While total and daily fecal output, daily DM intake, and ATTD of DM, organic matter (**OM**), and gross energy (**GE**) were not affected, CD had lower ($P < 0.05$) crude protein (**CP**) ATTD than did BPD and AMD, higher ($P < 0.05$) acid-hydrolyzed fat (**AHF**) ATTD than did AMD, with BPD not different ($P > 0.05$) from either, and lower ($P < 0.05$) TDF ATTD than either AMD or BPD. Beet pulp diet resulted in higher ($P < 0.05$) fecal scores compared to CD, and AMD was intermediate ($P >$

0.05). Fecal SCFA, acetate, and isobutyrate concentrations were greater (*P < 0.05*) for AMD and BPD than CD. The same relationship (*P < 0.05*) was noted with propionate and BCFA with AMD and CD, but BPD did not differ (*P > 0.05*) from either. Cats remained healthy on all treatments except for creatinine concentration, which are historically above reference ranges in this colony. A monadic acceptability test for AMD with only one coating (14.2% AHF, DM basis) resulted in poor and variable food intake, supporting our findings that an additional coating (16.7% AHF, DM basis) was needed to increase palatability.

Our third goal was to determine if avocado meal could be a dietary fiber source for canines. More often than not, AMD performed similarly to CD. As-is daily fecal output and fecal scores were greater (*P < 0.05*) for BPD than for AMD and CD. Cellulose diet had the greatest (*P < 0.05*) fecal DM, followed by AMD (*P < 0.05*), then BPD (*P < 0.05*). Fecal pH was lower *(P < 0.05*) for BPD and AMD than for CD. As with the feline study, only CP, AHF, and TDF ATTD were affected by the treatments. Avocado meal diet and CD had greater (*P < 0.05*) CP ATTD than did the BPD. In contrast, cats fed CD had greater (*P < 0.05*) AHF ATTD and lower (*P < 0.05*) TDF ATTD than cats fed AMD or BPD. In terms of fecal fermentative end-products, BPD resulted in greater (*P < 0.05*) concentrations of total SCFA, acetate, and propionate than did AMD and CD. Beet pulp diet also had lower (*P < 0.05*) levels of isovalerate, ammonia, and total phenols and indoles than AMD and CD. Fecal butyrate concentration was lower (*P < 0.05*) for CD than for AMD and BPD, but BPD had a greater (*P < 0.05*) concentration of valerate than did CD and AMD. Dogs remained healthy during the study and serum metabolites were within reference ranges for adult dogs among all dietary treatments, even though statistical differences were detected for a few serum metabolites. As with the feline study, monadic food acceptability

tests resulted in low consumption, indicating that additional fat and palatant were needed to increase acceptability of AMD (14.2% AHF, DM basis vs. 17.8% AHF, DM basis).

Based on the research in this study, avocado meal appears to be an acceptable dietary fiber source for canines and felines. It processes well within standard extrusion conditions of commercial pet foods and resulted in physiological effects similar to standard fiber sources for the pet food industry. Although acceptability of AMD was low prior to the second coating, a commercial pet food would likely not contain as much avocado meal as the diets tested herein (18.67%, as-is basis), possibly minimizing the less favorable food acceptability outcomes observed in our studies. More importantly, no detrimental effects on health status of the cats and dogs fed the AMD were observed, which does not support current safety concerns related to the consumption of avocado by domestic dogs and cats due to acute persin toxicity, at least not during a feeding period of 14-21 d evaluated herein.

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CHAPTER 1

INTRODUCTION

In the U.S., cats and dogs are mainly fed commercial complete and balanced diets, and dry extruded kibble diets are chosen more often by pet owners than other diet formats. These diets are formulated to meet the nutritional needs of cats or dogs and usually include a fiber source. The growing global human and pet populations often compete for similar food ingredients: however, the pet food industry seeks to maintain a sustainable food supply chain by utilizing coproducts from human food processing that can be nutritionally adequate for pet animals. One such ingredient is avocado meal, which is a byproduct of avocado oil processing. While it has been used for livestock feeds, many pet owners and veterinarians are advised against feeding avocados to pets because it is highly toxic. This anecdotal fallacy has kept avocado meal from formulation decks even though it may be a sustainable and functional ingredient in feline and canine diets.

Previous research on the effects of ingredients on the processing of extruded pet foods is sparse and limited to the extrusion of single ingredients (Murray et al., 2001). More information is available from research targeted for extruded foods for humans, but the experimental formulations are usually limited to a starch and fiber or protein source (Lue et al., 1991; Rinaldi et al., 2000; Sosa-Moguel et al, 2009). Often these products are not complete and balanced, which is a requirement of all pet foods. Experiment 1 begins to fill this knowledge gap by evaluating the processing changes needed to produce similar extrudates with different fiber sources (i.e. avocado meal, beet pulp, and cellulose) and by characterizing the physical and chemical attributes of those kibbles. It was hypothesized that the avocado meal-containing diet (**AMD**) would require moderate processing parameters and result in extrudates with physical

characteristics somewhere between beet pulp- and cellulose-containing diets (**BPD** and **CD**, respectively). We also expected the chemical composition of all three diets to experience minimal changes due to processing, expect for increases in soluble dietary fiber and decreases in insoluble dietary fiber.

The only published research testing avocado meal and other avocado oil processing by-products as animal feed/food ingredients has been done with rats (Naveh et al., 2002), sheep (Skenjana et al., 2006), and broiler chickens (van Ryssen et al., 2013). This ignores the need for novel fiber sources in the pet food market. Experiments 2 and 3 attempted to fill the knowledge gap by feeding an avocado meal diet to cats (Experiment 2) and dogs (Experiment 3) and comparing it to diets containing beet pulp or cellulose, which are standard dietary fiber sources commonly used in commercial pet foods. Each study assessed the effects of AMD on macronutrient apparent total tract digestibility (**ATTD**) to determine the adequacy of avocado meal as a dietary fiber source in feline and canine nutrition. Serum chemistry and complete blood count profiles were conducted to determine if avocado meal had any health implications for cats (Experiment 2) and dogs (Experiment 3). Our hypothesis was that AMD would have intermediate fermentability in comparison to BPD and CD, without negatively affecting macronutrient ATTD. We also expected AMD to be well tolerated and not cause signs of toxicity in blood profiles of cats (Experiment 2) and dogs (Experiment 3) fed this diet.

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CHAPTER 2

LITERATURE REVIEW

PET FOOD AND EXTRUSION

The pet food industry is fast-growing; global sales grossed 75.25 billion U.S. dollars in 2016 alone and represented 73% of the pet care and supplies sector (Phillips-Donaldson, 2017). Many trends have come and gone in the pet food market, but two trends are consistent: the popularity of extruded dry kibble products and the use of functional ingredients, which can provide specific benefits to the processing of pet foods and animal health.

Most published research addressing nutrient retention and extrudate physical characteristics investigates differing processing parameters and formulations. Lue et al. (1991) used corn meal and sugar beet fiber formulations to determine if screw speed and sugar beet fiber particle size had any impact on dietary fiber content, expansion, and starch gelatinization. They tested 4 corn meal:sugar beet fiber ratios (0:100, 10:90, 20:80, and 30:70), 2 screw speeds (200 rpm and 300 rpm), and 4 sugar beet fiber particle sizes (U.S. mesh sizes 10, 40, 120, and 200). All other parameters, including extruder settings, were kept constant. Those researchers found that expansion was significantly affected by the treatments; increased inclusion of sugar beet fiber and increased screw speed decreased extrudate diameter but increased extrudate length, whereas smaller sugar beet fiber particle size increased both extrudate diameter and length. Increased sugar beet fiber inclusion allowed for more air cell formation, but the air cells were smaller, thus affecting only extrudate length. The same relationship was seen with increased screw speed and air cell formation. The authors suggested that smaller sugar beet pulp particle size allowed for more air cells to form on the fiber, whereas larger particle size prevented

nucleation. In the same study, no differences ($P > 0.05$) in starch content or gelatinized starch were observed among treatments. Insoluble dietary fiber (**IDF**) decreased ($P < 0.05$) compared to the raw ingredients in the following treatment combinations: 10 mesh and 200 rpm, 10 mesh and 300 rpm, 120 mesh and 200 rpm, 120 mesh and 300 rpm, and 200 mesh and 300 rpm. In general, soluble dietary fiber (**SDF**) increased and total dietary fiber (**TDF**) decreased, but these differences were not significant ($P > 0.05$). The authors noted that a portion of IDF could be modified to SDF in the extruder (Lue et al., 1991).

Rinaldi et al. (2000) expanded upon this research using two soft wheat flour and wet okara formulations (33.33% or 40.00% wet okara). Okara is a by-product of the production of soy milk and tofu and is high in dietary fiber (> 50%) (Watanabe et al., 1984). They tested 4 extrusion conditions (i.e., low shear and low temperature, low shear and high temperature, high shear and low temperature, and high shear and high temperature) on radial expansion ratio, bulk density, breaking strength, and dietary fiber content. The different temperature treatments were achieved by altering the temperatures inside sections of the extruder barrel (low temperature: 40, 60, 125, 135, and 145 ºC; high temperature: 40, 60, 130, 155, and 170 ºC). Radial expansion ratio decreased ($P < 0.05$) as the inclusion of wet okara increased, which agrees with Lue et al. (1991) finding that increased fiber inclusion decreases the radial expansion ratio. This measurement also tended (P-value not given) to decrease at higher shear and temperature. Lower radial expansion was correlated with increased bulk density breaking strength (the amount of force needed to break the extrudate per gram, **g**), as decreased expansion results in less air contained within the extrudate, thus increasing the weight of the product, and thicker air cell walls, which makes the extrudate tougher and harder to break. Total dietary fiber remained unaffected ($P > 0.05$) by processing compared to the raw ingredients, except for two treatment

combinations. Wet okara included at 33.33% and extruded at low shear and low temperature and 40.00% wet okara extruded at high shear and low temperature had lower and greater ($P < 0.05$) TDF concentrations compared to the raw ingredients, respectively. On the other hand, more differences ($P < 0.05$) were noted in SDF and IDF. Soluble dietary fiber of the 40.00% wet okara products were all greater ($P < 0.05$) than the raw ingredient blend, and the 33.33% wet okara formulation differed ($P < 0.05$) at all parameter combinations compared to the raw ingredients except low shear and low temperature. In agreement with observed changes in SDF concentrations due to processing, IDF decreased ($P < 0.05$) for all 33.33% and 40.00% wet okara samples, except for the 40.00% wet okara low temperature samples (both low shear and high shear), which were not different ($P > 0.05$) from the raw ingredient blend. The IDF concentration of 33.33% wet okara low shear samples (both low and high temperature) were not different ($P > 0.05$) from each other but were higher ($P < 0.05$) than the 33.33% high shear samples (both low and high temperature). Of the significant 40.00% wet okara samples, IDF decreased ($P < 0.05$) as shear increased when temperature was high. Rinaldi et al. (2000) suggests that extrusion modified the structure of the product's dietary fiber and converted IDF to SDF. Even though Lue et al. (1991) did not observe the increase in soluble dietary fiber, it is possible that the fiber content of their raw blends was not high enough to note significance.

Sosa-Moguel et al. (2009) tested a corn flour:cowpea seed formulation (85:15). The blend was extruded at 3 different temperatures (150, 165, and 180 °C) and 3 moisture contents (15, 17, and 19%), though the paper presents data only for 15% moisture at 150 °C and 165 °C. They found that increased moisture content decreased ($P < 0.05$) extrudate expansion (data not shown), which is supported by the theory that water lubricates the extruder screw and decreases the interaction between the screw and product. Specific extrudate weight also was decreased (*P*

< 0.05) by higher temperatures and moisture contents. There was no difference ($P > 0.05$) in chemical composition, *in vitro* protein digestibility, and available lysine (following methods described in Booth, 1971) between the 15% moisture-150 °C condition and the 15% moisture-165 °C condition. Except for TDF, all starch and dietary fiber components (total starch, available starch, resistant starch, IDF, and SDF) for the 15% moisture-150 °C extrusion were higher ($P < 0.05$) than the 15% moisture-165 °C processing. These results support the findings of Rinaldi et al. (2000) which demonstrated that a starch-protein formula may behave similarly to a starch-fiber formula.

Unfortunately, no research has been published on the effects of extrusion processing on pet food formulations, which contain a wide ingredient deck supplying energy, protein, fat, vitamins, minerals, and carbohydrates, including dietary fiber. Dietary fiber sources are vital for supporting the health of pet animals, which in 2016 grew to 89.7 million cats and 94.2 million dogs in the U.S. (APPA, 2017). At the same time, the United Nations Department of Economic and Social Affairs Population Division forecasts the global human population will reach 8.5 billion by 2030 and 9.7 billion by 2050 (United Nations, 2017). These populations often compete for similar food ingredients, so it is vital to find novel and alternative ingredient sources that can be safely fed to cats and dogs. Ingredients for animal diets include by-products of the human food processing industry, which are often underutilized or destroyed. Thus, including these by-products in pet food formulations decreases production waste and competition with the human food industry, resulting in a more sustainable food supply chain.

DIETARY FIBERS IN FELINE AND CANINE DIETS

Different dietary fiber sources are usually characterized by their fermentability and solubility. Fermentability refers to how available a fiber source is to the microbiota in the gastrointestinal tract and the different products produced due to its degradation. Initial research in this area was done by Fahey et al. (1990) who found that by increasing the levels of beet pulp in a dry extruded kibble decreased macronutrient digestibility, including organic matter (**OM**), dry matter (**DM**), and acid-hydrolyzed fat (**AHF**). High fiber concentrations (greater than 7.5% DM beet pulp) also increased stool volume and frequency, as well as DM content. Ultimately, Fahey et al. (1990) determined that the moderately fermentable fiber (i.e., beet pulp) at a moderate level (7.5% DM) can optimize nutrient digestibility and minimize the stool volume and defecation frequency. Beet pulp is considered the gold standard dietary fiber source in foods for cats and dogs due to its optimal IDF:SDF ratio of approximately 70:30 (Fahey et al, 1990) and beneficial physiological effects in pet animals. For these reasons, beet pulp often is used as a positive fiber control in research evaluating novel dietary fiber sources for cats and dogs.

More recently, research has been done characterizing a variety of fiber sources and their impact on feline and canine physiology. Bueno et al. (2000) conducted a study with 28 adult female cats to better understand the impact of different dietary fibers on food intake and fecal short-chain fatty acids (**SCFA**). Their dietary treatments were fed for at least 15 days (**d**) and included a no-added fiber diet, a cellulose diet, a beet pulp diet, and a pectin/gum arabic diet. Cellulose was chosen as a non-fermentable, beet pulp as moderately fermentable, and pectin/gum arabic as easily and readily fermentable fiber sources. The four diets all had similar nutrient compositions except for the no-added fiber diet, which was higher in nitrogen free extract (35.8% as-is) and lower in total fiber (3.0% as-is) compared to the three fiber diets (26.6-30.1%

as-is and 8.4-8.8% as-is, respectively). The researchers found that the dogs bed the beet pulp and cellulose diets had greater ($P < 0.05$) food intake compared to the no-added fiber diet, but consumption of the pectin/gum arabic diet was lower than the no fiber diet. The increased food intake for cellulose and beet pulp did not result in significantly different body weight (**BW**) change, which was only observed as weight loss with pectin/gum arabic. These findings suggest that a diet using pectin/gum arabic as a single highly fermentable fiber source to reach a high total fiber content may not be advised for a maintenance diet due to the loss in BW as well as lower food and water intake and undesirable fecal consistency.

Those same researchers also measured absorption of SCFA in the large intestine by conducting a perfusion experiment at the end of the experimental period (Bueno et al., 2000). While they did not find treatment effects ($P > 0.05$) in total SCFA and propionate absorption, differences were seen in acetate and butyrate, both having a lower ($P < 0.05$) absorption in the dogs fed the cellulose diet than the beet pulp diet, with the no fiber and pectin/gum arabic diets being intermediate and not differing ($P > 0.05$) from the other dietary treatments. Bueno et al. (2000) suggest that a moderately fermentable fiber source, like beet pulp, is most ideal because cats might have developed both active and passive transport systems during the adaptation period to utilize the supply of SCFA. On the other hand, the no-added fiber, cellulose, and pectin/gum arabic-fed cats likely developed only passive transport systems during the adaptation (no fiber and cellulose because there few SCFA entering the colon; pectin/gum arabic because there was a large enough supply that there was no need to develop an active transport system) and were not equipped to transport the larger quantity delivered in the perfusion.

Barry et al. (2010) studied the impact of different fiber sources on microbial fermentation in the gastrointestinal tract of felines. The treatments in this study were cellulose,

fructooligosaccharides (**FOS**), and pectin, which were included in the diets at 4% as-is. As with Bueno et al (2000), cellulose was chosen because of its low fermentability and pectin for its high fermentability. Fructooligosaccharides are commonly used as a prebiotic and are rapidly and proximally fermented in the hindgut without altering digesta viscosity. The 4% inclusion level resulted in different TDF contents among the dietary treatments (7.9%, 6.7%, and 3.6%, DM basis, for cellulose, pectin, and FOS, respectively). The different dietary fiber sources also resulted in different amounts of SDF and IDF in the diets (cellulose = 6.5% IDF and 1.4% SDF, pectin = 4.6% IDF and 2.1% SDF, and fructooligosaccharides = 2.6% IDF and 1.0% SDF, DM basis). Male cats (n = 12) were fed for 20 d of adaptation and 10 d of total and fresh fecal collection. While food intake did not differ among treatments, crude protein (**CP**) apparent total tract digestibility (**ATTD**) tended ($P = 0.079$) to be lower for pectin (87.4%) than cellulose (90.5%) with FOS (88.0%) not differing from either ($P > 0.10$). Apparent total tract digestibility of AHF (92.5%) by cats fed pectin was lower ($P < 0.05$) than for cats fed cellulose (95.8%) or FOS (95.3%). Using a 5-point scale (1 = hard, dry pellets and 5 = pourable liquid), cellulose resulted in lower fecal scores (2.0) than FOS (2.8) and pectin (2.7), though daily fecal output (as-is and DM) and fecal DM were not impacted. Feces from cats fed the cellulose diet exhibited lower ($P < 0.05$) concentrations of ammonia and 4-methyl phenol compared to the other two treatments and lower ($P < 0.05$) fecal indole concentrations than pectin, while FOS was not different from either. Analysis of fecal SCFA and BCFA further supported the concept that cellulose is less fermentable than pectin, with FOS behaving like pectin. Cats fed the cellulose diet had lower ($P < 0.05$) fecal concentrations of butyrate, isobutyrate, isovalerate, valerate, and total BCFA than cats fed either pectin or FOS diets. These results suggest that pectin and FOS, in contrast to cellulose, support gastrointestinal tract health due to increased concentrations of

fermentative end-products and modulation of microbiota. Increased elimination of gastrointestinal microbiota in stools also may explain the trend towards a lower CP ATTD for the pectin diet.

A similar study was conducted by Howard et al. (2000) with 28 spayed adult female beagles. The researchers compared a diet containing a fiber blend (i.e., 6% beet pulp, 2% gum talha, and 1.5% FOS) to diets containing cellulose (6%), FOS (1.5%), and beet pulp (6%). Diets were fed for 30 d, followed by 5 d of total fecal and urine collection, before dogs were sacrificed and intestinal contents collected. Dietary treatments had no effect ($P > 0.10$) on food DM intake and on ATTD of most macronutrients. Dogs fed the fiber blend diet had greater ($P < 0.05$) nitrogen (**N**) in feces (expressed as g/d, DM basis) than dogs fed the FOS diet, while dogs fed cellulose and beet pulp had intermediate fecal N concentrations not differing from each other. However, there was no impact ($P < 0.05$) of the fiber source on fecal N excretion when expressed as a percentage of N intake or % of total N output, N digestibility (%), or N balance (g/d). Dogs consuming the cellulose diet exhibited in lower ($P < 0.05$) fecal microbial excretion (expressed as g microbial N/d) in contrast with the fiber blend, while FOS and beet pulp values were intermediates and did not differ from each other ($P > 0.05$). These findings suggest that fermentable fibers provide more available nutrients for fermentation by the hindgut microbiota of dogs when compared to a minimally fermentable fiber source like cellulose based on microbial N excretion in feces.

Similar to previous findings, Bosch et al. (2009) observed comparable results when they tested two diets containing either 8.5% cellulose (poorly fermentable) or a combination of 8.5% beet pulp plus 2% inulin (highly fermentable), as-is basis. These researchers found that the minimally fermentable diet caused the dogs to have higher DM content of fresh feces and

decreased total SCFA concentrations, as well as acetate and propionate concentrations, compared to the highly fermentable diet. These findings were expected as fermentable fiber sources are readily utilized by colonic bacteria, increasing the fecal concentrations of fermentative end-products such as SCFA, and may exhibit differences in concentrations of branched-chain fatty acids (**BCFA**), phenols, and indoles (Bosch et al., 2009).

In the same study, the impact of fiber fermentability on satiety was evaluated (Bosch et al., 2009). Satiety was assessed by offering a highly palatable diet to adult dogs for 20 minutes (**min**) at the end of the study (7 weeks, **wk**) and recording voluntary food intake. A dog that consumed more of a highly palatable diet was considered to be less satiated than a dog that ate less of the highly palatable diet. There was a trend ($P = 0.058$) for dogs fed the highly fermentable diet to have a lower voluntary food intake compared with the dogs fed the minimally fermentable diet. Blood samples were collected and analyzed for GLP-1 and PPY, which are appetite-related hormones. Those authors hypothesized that these hormones would be increased in dogs fed the highly fermentable diet, resulting in increased satiety. Unfortunately, there were no statistical treatment differences in the hormone concentrations, which the researchers suggest might be caused by a limited difference in soluble fibers consumed (as-is basis) between the highly fermentable and minimally fermentable diets (Bosch et al., 2009).

Dietary fibers are heterogeneous compounds and, as such, may result in different physiological effects and ATTD upon consumption. Fischer et al. (2012) conducted a study to evaluate how different levels of fiber fermentability impact nutrient ATTD in adult cats. The authors compared three different fibers sources to each other as well as to a diet that did not contain an added fiber source. The three fiber sources tested were beet pulp, wheat bran, and sugarcane fiber. These fibers were chosen because they differed in their IDF: SDF ratios (beet

pulp = 1.89:1; wheat bran = 5.91:1; sugarcane fiber = 21.40:1). All diets were formulated to have similar TDF contents (beet pulp diet = 25.6%, wheat bran diet = 24.0%, and sugarcane diet = 28.5% on a DM basis), except for the no fiber diet (11.6%).

A lower ($P < 0.05$) ATTD of DM, OM, and gross energy (**GE**) was observed by cats fed diets containing fiber sources in contrast to the no fiber diet. Total dietary fiber ATTD was affected ($P = 0.004$) by the fiber source, with the sugarcane fiber diet (11.4%) being less digestible than the beet pulp (31.6%) and wheat bran (22.7%) diets. The researchers hypothesized that this was caused by the different fermentabilities of the fiber sources, with sugarcane fiber being less fermentable than beet pulp and wheat bran. They also suggested that solubility impacts digestibility, which would explain why beet pulp and wheat bran were more digestible than sugarcane fiber. Including beet pulp in a diet also increased the moisture content of the feces, while including sugarcane fiber decreased moisture content; this further shows the impact of fiber solubility. Despite the similar IDF and SDF concentrations in the wheat bran and sugarcane fiber diets, all coefficients of ATTD differed (but were considered acceptable and were above at least 80%), except for starch and AHF. The researchers hypothesized that the latter was due to the heterogeneity in the composition of the different fiber sources (Fischer et al., 2012).

Inclusion of dietary fibers in pet foods may not only cause reduced nutrient ATTD, but also may affect food palatability and daily food intake. Fekete et al. (2001) tested graded-levels of apple pomace in a diet for cats. They believed that dried apple pomace, which is the by-product of processing apples for human consumption and "may contain the skin, peel, seed, and pulp" (AAFCO, 2018) would not affect palatability because of its sweet taste. Four different levels (0, 10, 20, and 40%, DM basis) were added at the expense of a basal diet consisting of

heat-treated, ground, whole rats, 0.8% salt, and 0.5% premix. As the inclusion of apple pomace increased, DM, crude fiber, and nitrogen-free extract contents of the diets also increased, while metabolizable energy (**ME**) and ether extract contents decreased (statistical differences were not reported). Nine neutered male cats were fed all diets using a phase system: they were fed the 0% inclusion diet during phase 1, 10% inclusion during phase 2, 20% inclusion during phase 3, and 40% inclusion during phase 4. Each phase consisted of 4 d of adaptation and 5 d of fecal collection. They found that ATTD of DM, OM, CP, and ether extract were decreased to varying degrees by addition of apple pomace. Greater statistical differences were notes between the 0 and the 20 and 40% diets ($P < 0.001$) for CP and ether extract ATTD than between the 0 and 10% diets ($P < 0.01$). The authors concluded that a rate of 40% inclusion of apple pomace might be too high due to the lower ATTD of ether extract (93.54%). Instead, they suggested that a 10 or 20% inclusion would be better since the ether extract ATTD (97.90% and 97.01% DM basis, respectively) were not as severely decreased (Fekete et al., 2001). Unfortunately, the researchers controlled for DM intake, which does not allow for any assessment of palatability or acceptability. Daily food intake or food refusals also were not reported. Furthermore, their design could not determine if the sweet taste or the lack of a bitter taste would support normal intake, since cats do not have both receptors needed to detect sweetness (Li et al., 2006), and potential treatment residual effects could not be addressed as well.

Apple pomace is just one example of a by-product of the human food (produce) industry that can be used as a dietary fiber source in diets for dogs and cats. Swanson et al. (2001) evaluated eight vegetable and fruit by-products [i.e., apple pomace, carrot pomace, flaxseed, a fruit blend (containing almond, nectarine, peach, and plum), grape pomace, pea hulls, pistachio fiber, and tomato pomace] and compared them to citrus pectin, psyllium, and cellulose in an *in*

vitro experiment. The fiber standards were chosen because of their fermentability profiles: highly (citrus pectin), moderately (psyllium), or minimally fermentable (cellulose). Canine fecal samples were utilized to determine the OM disappearance and production of SCFA *in vitro*. After 24 hours (**hr**) of fermentation, the three standards performed as expected, with cellulose having the lowest gas and SCFA production, citrus pectin the highest, and psyllium husk performing in between the two. Swanson et al. (2001) concluded from their data that the tested fruit and vegetable fibers could be grouped by fermentability, with flaxseed, carrot pomace, and apple pomace being among the highly fermentable, with grape pomace, pistachio, and the fruit blend among the lowest, and pea hulls and tomato pomace falling in between. In general, this follows the concept that a lower IDF:SDF ratio results in greater fiber fermentative profile. Swanson et al. (2001) also highlighted that other factors of fiber sources need to be considered. For example, the researchers cite the presence of carotenoids and antioxidants in some of the fiber sources, which may have additional health benefits to dogs besides supporting gastrointestinal health and changes in microbiota.

AVOCADO CHARACTERISTICS AND PROCESSING

Avocados (*Persea americana*) have become a popular fruit in human nutrition in recent years. According to the United States Department of Agriculture Economic Research Service, Americans consumed approximately 3.2 kilograms (**kg**) per person of avocados in the 2014/2015 census year (2016). This is a substantial increase from just 5 yr prior (2009/2010 census year) when Americans were only consuming 1.9 kg annually per person (USDA ERA 2016).

Different pigments in foods have been associated with specific compounds that may have health benefits to those who consume them. The main three pigments commonly found in

avocados are carotenoids, chlorophylls, and anthocyanins. Chlorophylls contribute green pigments, while anthocyanins are responsible for red pigments. Ashton et al. (2006) determined the concentrations of these compounds in four different parts of the avocado: the skin, the darkest green flesh (next to the skin), the middle of the flesh (denoted by a paler green color), and the flesh closest to the seed (a yellow color). The sections of the flesh were extracted from avocado (Hass variety) samples by using a plug and dividing the core up into thirds. Oil was extracted from the samples after freeze-drying using hexane as the solvent. Chlorophylls and carotenoids were extracted from all samples, including extracted oils, and quantified using high performance liquid chromatography. Anthocyanins were extracted and quantified only from the avocado skin.

Carotenoid and chlorophyll levels were higher in the skin than in any of the flesh sections (Ashton et al., 2006). Lutein was found in all samples at the highest level (8.0 μg/g in skin and 1.8 μg/g in the outer flesh, 0.4 μg/g in the median flesh, and 0.3 μg/g in the inner flesh) compared to all other measured carotenoids. Neoxanthin was the second most abundant carotenoid in the flesh samples (less than 0.4 μg/g), while β-carotene was the second most abundant in the skin (approximately 50% of the leutin concentration). Violaxanthin, α-carotene, antheraxanthin, and zeaxanthin were measured at low levels in all samples, as well as neoxanthin for skin and β-carotene for flesh samples. Chlorophyll-a levels were higher than chlorophyll-b levels in all flesh and skin samples (numerical values were not reported, only graphs). After oil extraction, the carotenoid and chlorophyll levels in the flesh decreased by 85-100% and 40-65%, respectively. This means that without the inclusion of avocado skin in avocado meal, carotenoid and chlorophyll levels would be expected to be low. On the other hand, the inclusion of avocado skin might increase those levels, as the skin retains roughly 72% of carotenoids and 71% of

chlorophylls. Cyanidin-3-O-glucoside was the most predominant anthocyanin in skin samples and all others were represented at extremely low levels in comparison. Anthocyanins were not measured in the oil extracted from the skin, so their retention could not be determined (Ashton et al., 2006).

Consumers have incorporated avocados into their diets in both the raw and processed forms (i.e. guacamole or cooking oil). Avocado oil also can be found in cosmetics and other personal care products. It's processing is similar to that of soybean oil, where the oil is either pressed out mechanically or extracted using a solvent. The impact of extraction method on avocado oil yield and quality was assessed by comparing four different extraction techniques: solvent extraction with acetone, solvent extraction with hexane, mechanical extraction using microwaving, and a mechanical-solvent extraction combination using hexane and microwaving (Moreno et al., 2003). The sample extracted using acetone as a solvent had the most undesirable characteristics of all the extracted avocado oils. It had the lowest extraction (12% yield) and had a very dark color, making it unsuitable for human consumption. Extraction with microwave and hexane resulted in the highest yield (97%), while hexane alone (54% yield) and microwave and manual squeezing (65.2%) resulted in intermediate yields. Hexane extracted oil had a yellow color compared to both microwave methods, which were green. Moreno et al. (2003) suggested that the green color came from free chlorophyll from chloroplasts. The hexane sample had higher viscosity than the microwave samples, but lower viscosity than the acetone sample. Fatty acid concentration was assessed using the acid value of each sample. The microwave samples had lower acid values, which means they had fewer oleic acids than the hexane and acetone samples; no information on the ideal acid value was reported, although values for all except the acetone extraction were acceptable compared to previously published results. They also had lower

peroxide values, which measures the rancidity of fats and oils. The fatty acid profiles of the four samples were significantly different for trans fatty acids, palmitic acid, palmitoleic acid, oleic acid, linoleic acid, and linolenic acid. The volatile compounds present in each sample differed as well, with only four compounds identified in the mechanical and microwave extraction, fifteen compounds in the hexane extraction and the microwave and hexane extraction, and twelve in the acetone extraction. A limitation of this paper is the researchers did not quantitatively describe what the ideal results would be. Even so, the acetone extraction was least acceptable because of oil color and low extraction yield. The researchers also believe that a combination of microwave and hexane extraction may be an acceptable method because of its high yield and acceptable fatty acid profile.

After avocado oil is extracted, the seed, peel, and defatted-pulp remain as by-products. In general, by-products are commonly fed to animals due to their nutritional profile and low cost. The previously mentioned avocado by-products are currently destroyed, but they may be suitable as a feed ingredient. Avocado meal is one such ingredient and is commercially defined as the processed residues from avocado oil processing that may include fat extracted pulp/flesh, the peel/skin, and the seed. At the moment, avocado meal is not commonly included in animal feeds and has been most used in ruminant nutrition. This is mainly due to limited information on the chemical composition and nutritional adequacy and little research available on avocado meal as a fiber source in monogastric diets.

AVOCADO MEAL AND OTHER AVOCADO BY-PRODUCTS

The first study to test avocado meal in an animal diet was done by Naveh et al. (2002), who extracted oil from freeze-dried and ground fruit and dried the remaining material, which

was referred to as defatted avocado pulp. They compared defatted avocado pulp (43.4% TDF, 15.8% moisture, 12.8% CP, 2.5% ash) to cellulose in two separate experiments. In Experiment 1, 40 Sprague Dawley rats were fed unlimited amounts of a semipurified diet containing either defatted avocado pulp or cellulose (target TDF content was 10%, as-is basis) for 28 d. At the end of the period, the rats were sacrificed for blood samples and tissue collection of the liver, cecum, kidney, and pancreas. Experiment 2 tested 3 different levels (30, 60, and 100 g/kg diet, as-is basis) of both defatted avocado pulp and cellulose with all diets containing 10 g/kg diet (as-is basis) of cholesterol to pair-fed rats.

Food intake, fecal output, weight gain, and feed efficiency were measured in both experiments (Naveh et al., 2002). In experiment 1, rats fed defatted avocado pulp consumed less food and produced lower amounts of feces compared to the cellulose-fed rats ($P < 0.05$). Feed efficiency was not different ($P > 0.05$). Experiment 2 showed increasing fiber levels of the same fiber source resulted in lower feed intake (g/d). There were no significant difference ($P > 0.05$) in feed intake between defatted avocado pulp and cellulose at the same inclusion level, except for 3% as-is basis (defatted avocado pulp = 11.9 g/d vs. cellulose = 10.8 g/d). The different results may be caused by the cholesterol supplementation in Experiment 2. Fecal output was lower ($P < 0.05$) in the defatted avocado pulp rats compared to the corresponding cellulose rats at all 3 inclusion levels. Body weight gain was not different ($P > 0.05$) at 3% inclusion, but was lower ($P < 0.05$) at the 6 and 10% inclusions for defatted avocado pulp. This was likely driven by the differences in SDF and IDF contents; defatted avocado pulp was higher in SDF than cellulose, and soluble fibers have been found to decrease BW and feelings of hunger in human patients (Krotkiewski et al., 1984). These results led to defatted avocado pulp having higher ($P <0.05$) feed efficiency than cellulose at all 3 inclusion levels.

Rat health also was monitored through blood and tissue samples. Defatted avocado pulp-fed rats had larger ($P < 0.05$) ceca relative to their body size in both experiments, with 10% inclusion greater ($P < 0.05$) than 3 and 6%. Plasma cholesterol levels for defatted avocado pulp rats were higher ($P < 0.05$) than cellulose rats in both experiments. The 6% defatted avocado pulp inclusion had higher ($P < 0.05$) plasma cholesterol compared to the 3% inclusion, with the 10% inclusion not different from either ($P > 0.05$). No treatment effect ($P > 0.05$) was seen in liver cholesterol. These findings are supported by Mensink et al. (1987), who found that a diet containing monounsaturated fat (24% of energy), which avocados are high in (approximately 9.5%), was linked to higher serum cholesterol levels than a diet containing less monounsaturated fat (9.3% of energy). Defatted avocado pulp is likely to contain some residual oil from the extraction process; it could contain some monounsaturated fatty acids and increase plasma cholesterol levels.

Skenjana et al. (2006) compared by-products from macadamia nuts to avocado meal. The avocado meal came from different avocado processing facilities in South Africa and the product was primarily comprised of avocados that had been processed for oil and could not be used in the fruit market. The chemical composition of this avocado meal was characterized as 9.5% CP, 51.82% neutral detergent fiber, 39.33% acid detergent fiber, 25.8% acid detergent lignin, and 0.38% acid detergent insoluble nitrogen, DM basis. They conducted an *in vitro* study to model how the avocado meal and macadamia by-products (macadamia oil cake and macadamia chips) were digested by rumen fluid collected from sheep. Organic matter digestibility of the avocado meal (54.3 ± 81.5% DM basis) was lower ($P < 0.05$) than macadamia oil cake (79.2 ± 199.8% DM basis), but higher ($P < 0.05$) than macadamia chips (29.2 ± 72.4% DM basis) and considered acceptable. Skenjana also used the avocado meal and macadamia oilcake in a 48 hr *in*

situ study with sheep and found that avocado meal had lower degradability of DM (67.1 ± 2.0%) and CP (61.7 ± 0.6%) in the diet (compared to 84.3 ± 6.3% and 92.2 ± 1.5% for macadamia oilcake, respectively). Given these findings, the researchers concluded that avocado meal should only be fed to animals that are not in high production phases due to the poor digestibility of avocado meal in comparison to the macadamia oil cake, as it could pose a challenge for the animals to easily obtain nutrients to meet their requirements for maintenance and production.

The most recent paper testing avocado meal in diets of animals was conducted by van Ryssen et al. (2013). Diets were fed to broiler chickens with increasing levels (0, 7.3, 14.7, 22.0, and 29.3%, as-is basis) of avocado meal that contained the seed, peel, and flesh from avocados that could not go into the human food market. Avocado meal (94.9% DM, as-is basis, 15.6% CP, DM basis, 6.3% crude fat, DM basis, and 34.9% crude fiber, DM basis) was added at the expense of maize (corn) meal in the diets. The researchers measured average daily gain, feed intake, and feed conversion efficiency for 28 d, and found that all three parameters decreased as the percentage of avocado meal in the diet increased.

In that study, birds were fed a commercial starter diet for 1-14 d before being assigned to treatments and receiving their treatment starter diet (15-21 d) and the finisher treatment diet (22-42 d). While the authors did not test the content of persin in the avocado meal or finished diets, they acknowledged that this toxin might be of concern for animal health upon consumption of avocado-derived ingredients. However, no signs of persin toxicity (i.e., swelling from fluid accumulation, difficulty standing or sitting upright, etc.) were observed in any of the 400 birds used in the study. An autopsy should have been done to confirm lack of damage to the myocardium, liver, and kidneys. The researchers suggested that persin was not an issue in their study because the avocado meal was dried, which has been shown to destroy toxins present in

foods (i.e., cyanogenic glycosides in cassava; Gomez et al., 1984). A limitation of this research was the use of corn meal as the ingredient for comparison. Avocado meal has a higher dietary fiber content than corn meal. The gradual increase in fiber content for the diets with graded levels of avocado meal could have caused the decreased performance. Dietary gross and metabolizable energy were not determined; this confounds the findings of this study since broiler chickens consuming higher levels of avocado meal likely also consumed less metabolizable energy than the birds fed the corn meal.

More research has been done on the effects of avocado seeds and their extracts. Imafidon et al. (2010) assessed the effects of avocado seed extract in an aqueous water solution on hypertension, because this treatment is commonly used in alternative medicine in Nigeria. Albino rats were used as the rodent model in a 4 wk long study, and hypertension was induced in 20 of the 25 animals by feeding a diet containing 8% sodium chloride, whereas the 5 other rats were fed a control diet without additional sodium chloride. Five hypertension-induced rats served as a hypertensive control and did not receive any avocado seed extract. The remaining 15 rats were split evenly into 3 groups receiving either 200, 500, or 700 milligrams (**mg**) of the aqueous seed extract per kg of BW. These dietary treatments were maintained for 4 wk, during which time BW was recorded daily. At the end of the study, systolic and diastolic blood pressures were recorded before the rats were sacrificed and the blood, heart, liver, and kidney were sampled for total cholesterol, high-density lipoproteins (**HDL**), low-density lipoproteins (**LDL**), and triacylglycerol concentrations.

Body weight gain was not affected by dietary treatment and food intake was not reported during the 4wk feeding study (Imafidon et al., 2010). The researchers successfully induced hypertension in the rats, as both systolic and diastolic blood pressures were significantly higher

for the hypertensive control (166.0 ± 1.0 mm Hg and 128.6 ± 6.1 mm Hg, respectively), hypertensive + 200 mg/kg avocado seed extract (145.0 ± 27.5 mm Hg and 76.6 ± 11.6 mm Hg, respectively), and hypertensive + 500 mg/kg avocado seed extract (125.0 ± 21.2 mm Hg and 66.6 ± 2.3 mm Hg, respectively) when compared to the non-hypertensive control (81.0 ± 3.05 mm Hg and 52.3 ± 4.3 mm Hg, respectively), except for diastolic blood pressure for hypertensive + 700 mg/kg avocado seed extract (91.4 ± 5.5 mm Hg and 56.4 ± 4.0 mm Hg, respectively). When compared to the hypertensive control, increasing supplementation of avocado seed extract resulted in reductions in systolic (200 mg/kg = 12.06%, 500 mg/kg = 24.7%, 700 mg/kg = 45.2%) and diastolic (200 mg/kg = 35.76%, 500 mg/kg = 51.32%, 700 mg/kg = 56.14%) blood pressure. The researchers suggested that the aqueous avocado seed extract contains potassium, which is found in avocados themselves and has been shown to lower blood pressure. In addition, the rats dosed with 500 mg/kg of the avocado seed extract had decreased total cholesterol, LDL, and triacylglycerol in plasma and all tissues and HDL in kidney and liver tissue only. The authors suggested that the decreased cholesterol might be caused by mono- and poly-unsaturated fatty acids, beta sitosterol, tocopherols, and/or glutathione, which may be present in the avocado seed extract. But at the highest tested supplementation of 700 mg/kg, rats had elevated levels of all four cholesterol fractions in plasma and nearly all tissues (excluding HDL in kidney and heart tissue) when compared to the non-hypertensive control and the hypertensive control. The researchers suggest that 700 mg/kg is above the upper limit of supplementation, even though blood pressure was still reduced at these levels of supplementation. There also was a possibility the 700 mg/kg supplemented rats developed hyperthyroidism, which would have induced these effects (Imafidon et al., 2010).

If avocado seed is included in avocado meal, it likely would be in the form of a flour and not an extract. Pahua-Ramos et al. (2012) produced the flour for their study by cutting, drying, and milling the seed through a US 20 mesh screen into a targeted particle size of 0.844 millimeter (**mm**) and a target moisture content of 4%, as-is. They measured phenolic compounds, antioxidants, and proximate analysis of the product and conducted feeding trials with mice to determine a median lethal dose and an effective dose to reduce cholesterol levels.

The researchers identified seven different phenolic compounds: protocatechuic acid (128.18 μg/g), kaemferide (107.42 μg/g), vanillic acid (28.67 μg/g), rutin (9.63 μg/g), syringic acid (2.51 μg/g), kaempferol (2.19 μg/g), and clorogenic acid (0.516 μg/g) (Pahua-Ramos et al., 2012). This equates to a total phenolic content of 292 mg gallic acid equivalents/g DM of seed, which is considered high for a by-product. The high measured antioxidant activity of 173.3 μmol Trolox equivalents/g DM of seed further indicates that avocado seed flour is a high value by-product. The proximate analysis suggested that avocado seed flour could have an effect on cholesterol due to fiber content (6.39% crude fiber, as-is basis, and 34.8% TDF, DM basis) and not due to fat content (4.38% ether extract, as-is basis). The discrepancy in the crude fiber and dietary fiber contents (approximately 28.14 percentage units) is due to insoluble and soluble fiber fractions that are not accounted for in the crude fiber measurement, such as pectins, gums, and some hemicelluloses. It also showed that avocado seed flour has low moisture (4.0%, as-is basis) and protein contents (4.75%, as-is basis) and high carbohydrate content (79.10%, as-is basis; Pahua-Ramos et al., 2012).

In this same study, the toxicity of avocado seed flour was analyzed in two stages, both measuring mortality, daily food intake, daily fluid intake, liver:BW ratio, and kidney:BW ratio (Pahua-Ramos et al., 2012). The first stage tested doses of 10, 100, and 1000 mg of avocado seed

flour/kg of BW and were compared to a control that did not contain any avocado seed flour. The second stage followed the same format, except doses of 1250 and 2500 mg/kg were tested against a control. Mortalities were seen only at the 2500 mg/kg dosage at a 100% level. Mice fed this dose also had decreased daily food and fluid intake, and liver and kidney weights were not recorded. Decreases in both liver and kidney weights were observed at the 1250 mg/kg dosage, but these differences were not significant ($P > 0.05$) compared to the control. The researchers did not comment on what compound or metabolite in the avocado seed flour could cause toxicity. Nevertheless, these findings suggest that a compound or combination of compounds in avocado seed extract negatively affected the liver and kidney at supra-physiological dosages. The calculated median toxic dose was 1767 mg/kg BW, which suggests that avocado seed extract is toxic only at higher doses.

The high cholesterol trials were designed similarly to the finding of Imafidon et al. (2010), except the doses were 125, 250, and 500 mg/kg BW. After 6 d of treatment, avocado seed flour did not affect plasma HDL or triglyceride concentrations. However, it resulted in lower ($P < 0.05$) total cholesterol, LDL, and the atherogenic index when compared to the hypercholesterolemic control mice (Pahua-Ramos et al., 2012). This agrees with results of the Imafidon et al. (2010) study and further supports the proposition that consumption of avocado seed flour or extract below toxic levels may decrease cholesterol levels.

PERSIN TOXICITY

As was previously mentioned, persin is a compound in avocados that many believe is toxic. Recent publications have advised against feeding avocado to avians (Lightfoot et al., 2008) and plant-eating reptiles kept as pets (Fitzgerald et al., 2008). Many case studies have been

published showing its detrimental effects on animal health. The first was published by Grant et al. (1991) in response to a goat herd becoming ill after consuming avocado leaves. Six of the twenty-one goats died as a result of eating the avocado leaves; three died within 3 d of starting the avocado leaf diet and the other three died within a month. The sick goats had labored breathing, decreased appetite, and lethargy. Since autopsies could not be done on these animals, the researchers decided to feed avocado leaves to four sheep via rumen fistulas. Sheep A was fed 25 g of fresh avocado leaves per kg BW per d until it died 5 d after beginning the trial. Before it died, this sheep had a visibly swollen submandibular gland. Upon conducting a necropsy, the researchers found lesions on the heart and congestion in the myocardium in addition to a notably fatty liver, the beginning stages of liver disease, and karyorrhectic cells in the spleen. Sheep B received 5.5 g of the same variety of avocado leaves per kg BW and was sacrificed 21 d later. This sheep showed the same changes in the liver and spleen as Sheep A as well as elevated blood urea nitrogen levels and inflammation of the myocardium. Sheep C and D were fed a different variety of avocado leaves due to a low supply at 2.5 g/kg for 32 d and 13 g/kg for 15 d followed by 25 g/kg for a second 15 d, respectively. Sheep C had a swollen myocardium, increased leukocytes, and an irregular heart rate. Sheep D was healthy enough at the conclusion of the study to not be sacrificed like Sheep B and C (Grant et al., 1991).

Given these results, Grant et al. (1991) concluded that avocado leaves may be poisonous to livestock if a large amount is consumed. Since Sheep C and D had less severe symptoms and were fed a different variety of leaves than Sheep A and B, they also suggest that some varieties could be more toxic than others. Unfortunately, a larger sample size would be needed to make a definitive conclusion. In addition, persin concentration was not determined in this study and,

therefore, no direct association or causation between persin (or any other compound) and toxicity signs in these animals can be drawn.

A second case study was conducted after nine ostriches in a flock of 120 died 96 hr after exposure to avocado trees for 24 hr (Burger et al., 1994). Eight of the nine birds had swollen necks and sat with their necks on the ground. The birds next exhibited dyspnea, which is difficulty breathing, and all birds died shortly after. Another bird exhibited similar signs and was euthanized before death. Anasarca, a type of swelling, was observed in the necks of three of the birds during necropsies. No pesticide residues were found in tissue samples from the proventriculus or the liver. Samples from the spleen, pancreas, intestine, lungs, and heart were also examined. The researchers found congestion in the lungs, swelling and hemorrhaging, cardiomyopathy, and nephrosis (kidney disease; Burger et al., 1994).

This case study spurred the conduct of two trials: one with New Hampshire hens and one with ostriches (Burger et al., 1994). Ten hens were used to determine if the foliage or the unripe avocado fruit was toxic and the consumption level needed to observe signs of toxicity. The doses were administered orally in a homogenized solution with 1% carboxymethyl cellulose and water. Food was withheld each day before supplementation. Four doses of foliage (5, 12.5, 23, or 25 g/kg BW) and two doses of unripe fruit (15 or 25 g/kg BW) were tested with 1% carboxylmethyl cellulose and water serving as the control. Cardiomyopathy and moderate kidney disease were seen for 25 g of foliage supplementation (n=1). Moderate kidney disease as well as lesions on the myocardium were observed in a hen fed 12.5 g foliage (n=1) and hens fed 25 g foliage (n=1). The same myocardium lesions were observed with 15 g of unripe fruit (n=1). Unfortunately, due to the low number of experimental units and variable response to treatments, no definitive

conclusions on whether fresh leaves or unripe fruit was more toxic could be drawn from this study (Burger et al., 1994).

Burger et al. (1994) also conducted an ostrich study with eight birds to compare the leaves and fruit of Hass and Fuerte avocado varieties to verify what was more toxic (Burger et al., 1994). Four ostriches did not receive any avocado fruit or leaves and the remaining birds were fed either the leaves or fruit of one of the varieties (n=1/trt). All four ostriches on an avocado treatment died during the study, with the bird that consumed leaves from the Hass variety dying only 3 d after the first daily dose. The other three ostriches received twice their normal dose on the fourth day and died on the fifth day of the study. These ostriches also exhibited general anasarca and edema in the proventriculus and pericardium. All four ostriches that consumed part of the avocado also exhibited cardiomyopathy. There were no definite conclusions from the trial, but the researchers suggested that the leaves from the Hass avocado variety seemed to be the most toxic (Burger et al., 1994).

The only published case study related to dogs and cats was published in the same year. Buoro et al. (1994) examined two dogs with potential toxicity at the University Veterinary Teaching Hospital (Nairobi, Kenya). Unfortunately, both dogs perished due to their illnesses; one of the dogs died before examination and the other died on the following day. The dogs lived on separate farms for about 1 yr and were described as "liking to eat avocados". Blood and tissue samples from one of the dogs were unavailable for analysis as the dog died before examination. Physical inspection of the other dog (while alive) found impaired heart function; decreased pulse, soft beating, cardiomegaly, difficulty breathing, and emaciation. Blood work showed high levels of leukocytes (20000 x 10^9/L; normal was 6000-17000/L), alanine aminotransferase (120 IU/L; normal was 21-102 IU/L), and alkaline phosphatase (180 IU/L; normal was 20-156 IU/L) and

normal levels of urea nitrogen and protein. High levels of protein were seen in ascetic fluid (4.0 g/100 ml; normal was less than 2.5 g/100 ml) and in the urine (75 mg/100 ml). A necropsy was done on both dogs. The hearts of both dogs were pale and exhibited damage to the myocardial fibers and decreased muscle tone. Swelling and fluid accumulation were seen in the hind legs, lungs, heart, and peritoneal cavity.

Given these results, the veterinarians believed both dogs died from congestive heart failure. Since the symptoms appeared quickly, they also believed the myocarditis was a secondary symptom. The exam and test results ruled out parasites and metabolic diseases, leading the veterinarians to believe that a toxin caused the symptoms. Since the dogs showed "a fondness for avocados" and the farms had the Fuerte variety on the property, they concluded that the dogs likely consumed toxic quantities of avocados. The veterinarians referenced the Grant et al. (1991) case study as support for their findings (Buoro et al., 1994). Unfortunately, their conclusions cannot be substantiated as a single subject does not provide enough data to eliminate individual variation and form sound conclusions. Furthermore, the veterinarians also never tested the avocados on the farm, documented how recently the dogs consumed avocados and the quantity consumed, and did not identify or suggest the toxic compound in the avocados. They also only compared their findings to case studies where avocado leaves were fed to goats; it is not appropriate to assume avocados are toxic to dogs when only the leaves of the avocado tree were fed to goats.

Oelrichs et al. (1995) took a chemical approach to assessing the toxicity of avocado leaves. They fed ground, freeze-dried leaves from a Guatemalan variety of avocados and isolated fractions of persin from the same leaves to white lactating Quakenbush mice, either by providing 20 g of commercial food at a 5% inclusion or by orally dosing isolated fractions or compounds

of persin. The dams were monitored along with eight nursing pups for 3-7 d afterwards; any negative changes in the growth of the pups was associated with underlying health consequences. Once the dams were sacrificed, their hearts and mammary glands were removed and preserved for microscopic observations.

The researchers were able to confirm the chemical structure of persin as (Z,Z)-1-acetyloxy-2-hydroxy-12,15-heneicosadien-4-one (1) and isolate persin from the Guatemalan avocado leaves at a 1% yield (Oelrichs et al 1995). They were able to test both the S and R isomers as well as a mixture of the two, and found that the S isomer is inactive and the mixture of the isomers was not as toxic as the pure R isomer. Oelrichs et al. (1995) also observed that dams could survive doses of 60-100 mg of persin/kg of BW with detrimental, but not life-threatening, effects to the mammary gland. But doses higher than 100 mg/kg caused deaths of myocardium fibers and fluid accumulation in the lungs (Oelrichs et al., 1995).

Ali et al. (2010) also conducted an avocado toxicity study, but this time utilized twenty rabbits to determine the toxic dose of persin. The rabbits were fed an undetermined amount of fresh avocado leaves in the evening and assessed the following day. Eleven rabbits died overnight and four more died later that day. The nine rabbits who survived were anorexic, depressed, and had difficulty breathing. Serum was collected post-mortem and showed hypernatremia, hypochloremia, and hypophosphatemia. The researchers hypothesized that the low phosphorus level was related to the anorexic behavior of the rabbits. The high sodium level may have influenced the fluid accumulation in the pericardium. The gallbladder was swollen due to accumulated bile. The livers of these animals were generally swollen with many necrotic lobes. Ali et al. (2010) concluded that avocado leaves are toxic to rabbits and should not be fed to them.

CONCLUSION

Despite anecdotal claims that avocados are toxic to dogs and cats, avocado meal may be a suitable dietary fiber source for the pet food industry. Previous research has suggested that avocado meal is a moderately soluble and fermentable fiber source that has not caused any well-documented health cases. It may fit a market for novel fiber sources that are more "label friendly". As the pet population continues to rise, more fiber sources will be needed to produce enough food for these animals. Work is needed to verify whether avocado meal is an appropriate fiber source and is not toxic to dogs and cats so that companies can be confident in formulating with it and pet owners inclined to purchase foods containing it.

THESIS OBJECTIVES

The purpose of this thesis was to assess the use of avocado meal as a dietary fiber source in diets of domestic felines and canines. Limited research on avocado meal as a feed ingredient has been done with rats, sheep, and roosters, but not cats or dogs. We hypothesized that avocado meal would have an intermediate fermentative profile in comparison with cellulose and beet pulp, when incorporated as a fiber source in complete and balanced diets. Avocado meal was not expected to negatively affect macronutrient ATTD, fecal score, or fecal output, and levels of persin in this ingredient, if present, would not be detrimental to canine or feline health.

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CHAPTER 3

DESCRIPTIVE ANALYSIS OF THE EFFECTS OF FIBER SOURCE ON PROCESSING SETTINGS, NUTRIENT COMPOSITION, AND PHYSICAL CHARACTERISTICS OF EXTRUDED HIGH FIBER PET FOODS

ABSTRACT

Avocado meal is the by-product after avocado oil extraction from the pulp and may include defatted pulp, peel, and seed. It has been tested in rats, sheep, and broiler chickens, but never in cats or dogs. The goal of this experiment was to determine the chemical composition of avocado meal, compare the processing requirements of an avocado meal-containing diet (**AMD**) for cats and dogs to diets containing either beet pulp (**BPD**) or cellulose (**CD**), analyze the chemical composition of all processing stages for AMD, BPD, and CD, and examine physical characteristics of AMD, BPD, and CD extrudates after the extruder knife, dryer, and coater. The diets were formulated to meet the 2016 AAFCO nutrient profiles for dogs and cats with similar nutrient targets (~15% total dietary fiber, **TDF**). Composite samples were taken at all processing stages for the three diets. Avocado meal was low in crude protein [**CP**; 11.5%, dry matter (**DM**) basis] and acid-hydrolyzed fat (**AHF**; 9.1%, DM basis), with moderate amounts of TDF (37.4%, DM basis), insoluble dietary fiber (**IDF**; 27.0%, DM basis), and soluble dietary fiber (**SDF**; 10.4%, DM basis). Preconditioner and extruder settings were modified to achieve similar bulk densities, with BPD and CD more similar than AMD. Nutrient composition was mainly unaffected by processing, except for decreases in TDF after extrusion. Avocado meal diet and BPD exhibited greater expansion and lower hardness than CD. Removal of moisture in the dryer decreased hardness and energy to compress AMD and BPD extrudates, but not for CD. This

research suggests that complex matrices high in dietary fiber have varying effects on processing requirements and physical characteristics, but similar relationships are seen to varying degrees in nutrient composition. As such, these results suggest that processing may have little effect on physiological responses when these diets are fed to dogs and cats. However, variations in the physico-chemical properties of the extrudates may impact diet acceptability.

INTRODUCTION

There are few publications about the effects of processing on the nutrition of commercially extruded pet foods, but more research has been conducted with extruded foods for humans. In most cases, insoluble dietary fiber decreases ($P < 0.05$) after the extrusion process (Lue et al., 1991; Rinaldi et al., 2000). There is also some research suggesting that soluble dietary fiber increases ($P < 0.05$) (Rinaldi et al., 2000). More extreme processing conditions, such as faster extruder screw speeds and higher temperatures, result in greater differences in the chemical composition of the extrudate. Sosa-Moguel et al. (2009) found that extruding a corn/cowpea formula at a higher temperature (150 ºC vs. 165 ºC) resulted in lower ($P < 0.05$) IDF (3.9% vs. 2.9%) and higher ($P < 0.05$) SDF (2.1% vs. 2.8%). More extreme extrusion conditions are also known to change physical characteristics of extrudates. Rinaldi et al. (2000) tested a combination of two temperature profiles (low: 40, 60, 125, 135, and 145 ºC and high: 40, 60, 130, 155, and 170 ºC) with two shear levels (i.e., low and high) resulting in the following processing conditions: high shear-high temperature, high shear-low temperature, low shear-high temperature, and low shear-low temperature. They found that radial expansion ratio decreased and bulk density increased in treatment combinations with high shear or high temperature and

greatest with high shear and high temperature. Lue et al. (1991) also found that higher screw speeds (300 rpm vs. 200 rpm) produced products with smaller air cells.

While this knowledge is valuable to manufacturers of extruded pet food, the formulas used in the previously mentioned studies contained few ingredients, mainly carbohydrates. On the other hand, food for dogs and cats has a complex dietary matrix because it is the animal's only source of nutrition and, as such, needs to fulfill their daily nutritional requirements. A gap exists in the knowledge of how extrusion impacts the nutrient composition of multifarious formulas, such as those used in the pet food industry.

The objective of this study was to assess the impact of fiber source on the physico-chemical properties of extruded complete and balanced diets (i.e. AMD, BPD, and CD) for felines and canines. Diets containing beet pulp or cellulose were produced for comparison due to their popularity in commercially available extruded diets. Our hypothesis was that extrusion processing would decrease the ratio of insoluble dietary fiber to soluble dietary fiber (**IDF:SDF**), but all other nutrients would be minimally affected. We also hypothesized that extrudates of AMD and BPD would have greater radial expansion and lower bulk density due to their higher SDF content.

MATERIALS AND METHODS

Diets and Processing Parameters

Diets were formulated with either avocado meal (Green Source Organic Natural Extracts, Boynton Beach, FL), beet pulp, or cellulose. All ingredients, excluding avocado meal, were procured at Lortscher Animal Nutrition (Bern, KS). Formulations were designed to meet similar nutrient targets and are listed in **Table 3.1**. They were

formulated to meet the AAFCO (2016) nutrient profiles for adult cats and dogs with the following nutrient targets: 32% crude protein (**CP**), 12% crude fat, 10% total dietary fiber (**TDF**), 5% ash, and 10% dry matter (**DM**). The dry blends were mixed and ground to a uniform particle size by Lortscher Animal Nutrition, Inc. (Bern, KS) and choice white grease was sourced from the University of Illinois Feed Mill (Champaign, IL). Diets were produced in the Bioprocessing and Industrial Value Added Products Center at Kansas State University (Manhattan, KS; **Figure 3.1**) to a targeted bulk density off-extruder of 400 g/L. Raw material was hand-dumped into the screw feeder of a Wenger DDC2 preconditioner (Sabetha, KS). From there, preconditioned material directly entered a X-20 single screw Wenger extruder (Sabetha, KS) with the following screw configuration: inlet single flight screw, single flight screw, small shear lock, single flight screw, small shear lock, single flight screw, medium shear lock, double flight screw, large shear lock, and a double flight cone. At the end of the screw was a die plate with 4 millimeter (**mm**) circular dies and a 6 bladed knife. Extrudates were pneumatically conveyed to a Wenger 4800 series dryer/cooler. The product underwent two passes through the dryer for 8 minutes (**min**) each at 107 ºC and one pass through the cooler at ambient air temperature (21 ºC). Finally, extrudates were coated with choice white grease (University of Illinois Feed Mill, Champaign, IL) with a Wenger-made double ribbon mixer (Sabetha, KS) at a constant temperature of 60 ºC. Product was packaged directly into bags in boxes for transport.

Sample Collection

A representative sample of avocado meal was taken from the lot used in producing the AMD. Two samples were taken during stable extrusion conditions at five different stages for all

three experimental diets (i.e., AMD, BPD, and CD): raw product (**R**), after the preconditioner (**P**), after the knife of the extruder barrel (**E**), after the dryer (**D**), and after the coater (**CO**). Stable processing conditions were determined by measuring bulk density every 20 min and making adjustments accordingly. Samples were frozen in a -20 °C freezer until further analysis.

Chemical Analyses

Samples were pooled by processing stage for macronutrient analysis. The samples from the preconditioner and after the extruder die and knife were dried in a 55 °C forced-air oven to remove excess moisture before grinding. All samples were ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) with a 10-mesh size screen (2 mm). Each analysis was done in duplicate, allowing a 5% error between duplicates; otherwise, the analyses were repeated. Dry matter and organic matter (**OM**) were analyzed according to AOAC methods (2006; methods 934.01 and 942.05, respectively). Acid-hydrolyzed fat (**AHF**) was analyzed following procedures described by the American Association of Cereal Chemists (1983; method 30-14) and Budde et al. (1952). Total CP content was determined by measuring total nitrogen content using a LECO TruMac (model 630-300-300, Saint Joseph, MI) following the official AOAC method (2006; method 992.15). Gross energy (**GE**) was measured using a Parr 6200 calorimeter (Parr Instrument Company, Moline, IL). Total dietary fiber, SDF, and IDF were determined using methods published by Prosky et al. (1992), except for the avocado meal ingredient, which followed AOAC Method 991.43.

Physical Analyses

Ten kibbles per processing stage and dietary treatment were weighed individually on an analytical scale (model #AG104, Mettler Toledo, Switzerland). Measurements of radial diameter and piece length (**Figure 3.2**) were taken using 0-150 mm digital calipers (model #01407A,

Neiko Tools, China). Piece volume, piece density, sectional expansion index (**SEI; Figure 3.2**), and specific length were calculated using **Equations 3.1-3.4**, respectively. Values for each processing stage and dietary treatment were averaged together and sample standard deviation was calculated.

Equation 3.1: Piece volume formula

$$Piece\ volume\ (cm^3) = \pi * (Piece\ diameter\ in\ cm)^2 * \frac{(Piece\ length\ in\ cm)}{4}$$

Equation 3.2: Piece density formula

$$Piece\ density\ \left({}^{g}/_{cm^3}\right) = \frac{Piece\ mass\ in\ g}{Piece\ volume\ in\ cm^3}$$

Equation 3.3: Sectional expansion index (SEI) formula

$$SEI = \frac{(Piece\ diameter\ in\ mm)^2}{(Die\ diameter\ in\ mm)^2}$$

Equation 3.4: Specific length formula

$$Specific\ length\ \left({}^{mm}/_{g}\right) = \frac{Piece\ length\ in\ mm}{Piece\ mass\ in\ g}$$

Texture was analyzed with a TA HD *plus* (Texture Technologies Corporation, Scardsale, NY) with a 30 kg load cell and a 50.8 mm cylindrical probe (TA-15). The speed settings were 2 mm/s (pre-test), 1 mm/s (test), and 10 mm/s (post-test). Ten kibbles per processing stage and diet were compressed to 50% strain. Texture Expert Exceed software (Stable Micro Systems, Godalming, England) was used to analyze graphs from the texture analyzer. Peak force (Newton; **N**) was recorded as the greatest force measurement of each sample and energy required for 50%

compression (Newton x millimeter; **Nxmm**) was calculated from the area underneath the curve. This method was slightly modified from Dogan et al. (2007). Duplicates for each diet and processing stage combination were averaged together and standard deviation was calculated.

RESULTS AND DISCUSSION

Ingredient composition of experimental diets are reported in **Table 3.1**. Experimental diets were formulated to have similar ingredient composition, except for the source of dietary fiber (i.e., avocado meal, beet pulp, and cellulose). Beet pulp and avocado meal were added at the expense of cellulose and by removing portions of chicken by-product meal and brewer's rice to target similar nutrient composition among the dietary treatments. The AMD contained less added choice white grease because the avocado meal ingredient was higher in fat than were BPD and CD. Although off-extruder bulk density was lower than targeted, the dietary treatments were similar to each other with BPD (378.9 g/L) and CD (370.0 g/L) being the most similar and AMD (345.2 g/L) lower than the other treatments.

The chemical composition of the avocado meal ingredient (**Table 3.2**) was characterized by high DM (94.1%) and OM (91.0%, DM basis) concentrations and moderate TDF concentrations of 37.4%, with soluble and insoluble fractions of 10.4% and 27.0%, DM basis, respectively. Crude protein (11.5%, DM basis) and AHF (9.1%, DM basis) concentrations were relatively low.

Most processing parameters were altered during the manufacturing of AMD, BPD, and CD to result in diets with similar physico-chemical properties and to be adequate for feline and canine feeding trials. Only preconditioner cylinder speed and extruder steam flow were constant for the dietary treatments (**Table 3.3**). Raw material feed screw rate, preconditioner steam flow

rate, and preconditioner choice white grease flow rate were similar for BPD and CD, but lower for AMD. Preconditioner water flow rate was similar among the 3 diets, with BPD (11.2 kilograms/hour, **kg/h**) having the highest, CD (9.6 kg/h) the lowest, and AMD (10.7 kg/h) intermediate. The CD (94.1 ºC) had the highest preconditioner discharge temperature, followed by BPD (82.3 ºC) and AMD (59.0 ºC). Extruder shaft speed for CD (378.4 rotations per minute, **rpm**) was lower than for AMD (410.2 rpm) and BPD (407.3 rpm). The AMD had higher extruder operational torque (65.5%), zone 2 temperature (78.3 ºC), and die pressure (355.0 gauge pounds per square inch, **psig**) and lower extruder knife speed (2693.3 rpm) and zone 3 temperature (80.7 ºC) than BPD (53.6%, 70.3 ºC, 334.3 psig, 3047.0 rpm, and 88.1 ºC, respectively) and CD (55.1%, 71.3 ºC, 328.6 psig, 3041.0 rpm, and 91.4 ºC, respectively). The BPD had higher extruder water flow rate (7.8 kg/h) and die temperature (127.8 ºC) than the other two treatments (AMD = 1.7 kg/h and 117.7 ºC, respectively; CD = 0.8 kg/h and 114.9 ºC, respectively). These results indicate that diet formulation has an impact on the processing conditions needed to achieve similar physical and organoleptic characteristics among the extruded diets. Particularly, the higher intrinsic fat content of AMD appears to affect early stages of processing the most, resulting in lower steam injection during the preconditioner phase (approx. 50% lower compared to BPD and CD) and, consequently, lower discharge temperature at this processing stage (23-35 ºC lower than BPD and CD, respectively).

Extrusion processing had varying effects on the chemical composition of the experimental diets (**Table 3.4**). Dry matter was fairly consistent except for P and E stages, which were numerically lower. This was expected because of the water and steam additions at these stages. Organic matter and ash were also minimally affected. Acid-hydrolyzed fat was slightly higher after the P stage compared to the raw materials for BPD and CD, as these treatments had

choice white grease added at P. The AMD AHF was not affected because no choice-white grease was added at P. This concentration increased for all treatments in the CO stage, which again was expected. Crude protein concentration fluctuated during processing differently among the dietary treatments, although CO had a lower CP content than R for all treatments. This was expected as the additional fat added at the CO step proportionally dilutes the concentrations of other nutrients. In general, CP remained constant with up to a decrease of 4 percentage points in CO. Total dietary fiber was affected during the processing stages, with a trend for decreasing TDF at each stage. These results do not agree with previously published data. Lue et al. (1991) found that TDF and SDF were not affected ($P > 0.05$) by the extrusion process, but IDF was lower ($P < 0.05$) depending on the particle size of the fiber source in their corn meal-sugar beet fiber extrudates. Rinaldi et al. (2000) observed similar relationships when extruding soft wheat flour-wet okara extrudates; IDF decreased and SDF increased compared to the raw materials for all of their treatments. They only noticed two instances were TDF changed: TDF decreased when 33.33% wet okara was extruded under low shear-low temperature conditions and increased when 40% wet okara was extruded under high shear-low temperature conditions. Sosa-Moguel et al. (2009) also agreed with previous research in that higher extruder temperatures (165 °C vs. 150 °C) decreased IDF and increased SDF ($P < 0.05$) with no effect on TDF ($P > 0.05$). Our formulations were more complex than those tested in the mentioned studies, so there may be some ingredient or nutrient interactions affecting our results. Gross energy was not affected by processing until CO for all dietary treatments, which was expected due to the increase in fat content.

Average physical and organoleptic characteristics are described in **Table 3.5**. Piece mass was similar during the processing stages and among experimental diets, with AMD (E = 0.14 ±

0.01 g; D = 0.13 ± 0.02 g; CO = 0.13 ± 0.02 g) and BPD (E = 0.14 ± 0.01 g; D = 0.13 ± 0.01 g; CO = 0.15 ± 0.02 g) being slightly heavier than CD (E = 0.12 ± 0.01 g; D = 0.10 ± 0.01 g; CO = 0.12 ± 0.01 g). Diameter for BPD (E = 7.0 ± 0.2 mm; D = 6.6 ± 0.4 mm) and CD (6.8 ± 0.4 mm; D = 6.3 ± 0.3 mm) both decreased at D, while AMD (E = 7.3 ± 0.4 mm; D = 8.4 ± 0.7 mm) increased and had a larger standard deviation. Piece lengths (6.0 – 6.9 mm) between the diets were similar, which was expected because knife speed was modified to achieve similar lengths. The differences observed in piece diameter translated to greater piece volume for AMD (E = 0.27 ± 0.03 cm^3; D = 0.35 ± 0.09 cm^3; CO = 0.31 ± 0.05 cm^3) compared to BPD (E = 0.25 ± 0.02 cm^3; D = 0.22 ± 0.04 cm^3; CO = 0.24 ± 0.05 cm^3) and CD (E = 0.24 ± 0.03 cm^3; D = 0.20 ± 0.03 cm^3; CO = 0.21 ± 0.04 cm^3). This also caused piece density for BPD (E = 0.54 ± 0.04 g/cm^3; D = 0.61 ± 0.07 g/cm^3; CO = 0.66 ± 0.13 g/cm^3) and CD (E = 0.49 ± 0.05 g/cm^3; D = 0.51 ± 0.06 g/cm^3; CO = 0.57 ± 0.07 g/cm^3) to be greater than AMD (E = 0.53 ± 0.05 g/cm^3; D = 0.39 ± 0.06 g/cm^3; CO = 0.42 ± 0.06 g/cm^3). We also observed that the AMD (E = 3.3 ± 0.3; D = 4.5 ± 0.8; CO = 4.2 ± 0.6) had greater SEI compared to BPD (E = 3.1 ± 0.2; D = 2.7 ± 0.3; CO = 2.7 ± 0.4) and CD (E = 2.9 ± 0.3; D = 2.5 ± 0.3; CO = 2.5 ± 0.3) and was caused by the greater piece diameter for AMD. Specific length for AMD (E = 4.6 ± 0.3 mm/g; D = 4.7 ± 0.3 mm/g; CO = 4.7 ± 0.3 mm/g) and BPD (E = 4.8 ± 0.2 mm/g; D = 4.9 ± 0.1 mm/g; CO = 4.6 ± 0.3 mm/g) were very similar with CD (E = 5.7 ± 0.2 mm/g; D = 6.4 ± 0.2 mm/g; CO = 5.7 ± 0.3 mm/g) higher than both and was driven by the small differences in piece mass across dietary treatments. Kibble hardness was similar among all three diets at E (AMD = 59.6 ± 10.6 N; BPD = 59.9 ± 4.8 N; CD = 50.8 ± 10.7 N). The AMD (D = 42.2 ± 15.8 N; CO = 44.5 ± 13.4 N) and BPD (D = 27.4 ± 9.0 N; CO = 33.7 ± 6.1 N) had lower kibble hardness at D and CO stages compared to the E stage; however, these measurements varied. Mazumder et al. (2007) found that corn extrudates with a

higher moisture content had higher maximum force and greater energy to compress and concluded that the same formula would appear less crisp if produced at a high moisture content. On the other hand, CD had harder kibble at D and E stages compared with AMD and BPD. The differences seen between CD and AMD and BPD are likely due to the amount of SDF and IDF in the three products. The CD had a greater proportion of IDF than either AMD or BPD, which caused the internal structure to be more solid, thus requiring more energy to break. Our results agree with those of Yanniotis et al. (2007), who showed that cornstarch-wheat fiber (high IDF) extrudates had smaller air cells, decreased porosity, and increased hardness compared to cornstarch-pectin (high SDF) extrudates. Finally, energy to compress a kibble decreased throughout the process; however, CD (E = 124.1 ± 9.6 Nxmm; D = 89.5 ± 14.8 Nxmm; CO = 77.7 ± 12.6 Nxmm) required greater energy to compress in all stages in contrast to AMD (E = 77.9 ± 13.2 Nxmm; D = 39.2 ± 14.4 Nxmm; CO = 36.3 ± 5.3 Nxmm) and BPD (E = 70.2 ± 11.8 Nxmm; D = 16.1 ± 6.6 Nxmm; CO = 20.0 ± 6.7 Nxmm).

CONCLUSION

The research in this chapter shows that processing of extruded diets mainly affects IDF and SDF in terms of chemical composition, and different fiber sources in a high-fiber diet formulation for felines and canines will alter the processing requirements to achieve similar physical and organoleptic characteristics. A similar relationship of increasing SDF and decreasing IDF after the extrusion step was observed among the three diets, although results varied among different dietary fiber sources and proportions of each dietary fiber fraction. All kibbles had similar piece masses and longitudinal expansion, but slight differences were seen in radial expansion, which translated to differences in piece density, piece volume, and sectional

expansion index. Dietary fiber fractions also affected texture analysis; AMD and BPD showed a similar relationship of decreasing peak force at the dryer and increasing slightly after the addition of fat, while CD peak force increased at each stage. There were also periods of surging during the extrusion of all three diets, which could influence the measurements and was depicted in the fluctuating, high standard deviation.

One main takeaway from this research is the relationship between processing, ingredient composition, and nutrition. There are many gaps in the pet food industry's knowledge base of how processing and nutritionally balanced formulations affect each other and more research is needed to optimize pet food manufacturing.

TABLES AND FIGURES

Table 3.1: Ingredient composition of extruded dietary treatments containing select dietary fiber sources.

	Treatments[1]		
Ingredient, % as-is	AMD	BPD	CD
Chicken by-product meal	31.3	31.7	33.3
Brewer's rice	28.4	28.8	33.5
Choice white grease	5.7	8.0	8.0
Corn gluten meal	8.1	8.2	8.2
Whole corn	4.1	4.1	4.1
Avocado meal	19.8	0.0	0.0
Beet pulp	0.0	16.6	0.0
Cellulose	0.0	0.0	10.3
AFB bioflavor B22006	0.94	0.89	0.89
Salt	0.51	0.50	0.50
Potassium chloride	0.46	0.45	0.45
Taurine	0.20	0.20	0.20
Mineral premix[2]	0.18	0.18	0.18
Vitamin premix[3]	0.18	0.18	0.18
Choline chloride	0.13	0.13	0.13

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet

[2] Provided per kilogram of diet: 37.7 mg manganese ($MnSO_4$), 636.8 mg iron ($FeSO_4$), 31.3 mg copper ($CuSO_4$), 0.02 mg cobalt ($CoSO_4$), 345.5 mg zinc ($ZnSO_4$), 3.0 mg iodine (KI), and 0.9 mg selenium (Na_2SeO_3).

[3] Provided per kilogram of diet: 18000 IU vitamin A (retinyl acetate), 2700 IU vitamin D3, 144 IU vitamin E (DL-α tocopherol acetate), 3.5 mg vitamin K, 30.6 mg thiamin, 30.6 mg riboflavin, 54.7 mg pantothenic acid, 124.2 mg niacin, 30.6 mg pyridoxine, 0.1 mg biotin, 1.1 mg folic acid, and 1.2 mg vitamin B12 (mannitol).

Table 3.2: Chemical composition of avocado meal ingredient.

Item	
Dry matter, %	94.1
----- % DM basis -----	
Organic matter	91.0
Ash	9.0
Acid hydrolyzed fat	9.1
Crude protein	11.5
Total dietary fiber	37.4
Soluble dietary fiber	10.4
Insoluble dietary fiber	27.0
Gross energy, kcal/g	4.9

Table 3.3: Average Wenger X-20 single screw extruder processing conditions for dietary treatments containing select dietary fiber sources.

	Treatments[1]		
Measurement	AMD	BPD	CD
Raw material feed screw speed, rpm	13.3	15.2	15.2
Preconditioner cylinder speed, rpm	400.0	400.0	400.0
Preconditioner steam flow, kg/h	6.5	13.9	13.1
Preconditioner water flow, kg/h	10.7	11.2	9.6
Preconditioner choice white grease flow, kg/h	0.0	22.0	24.4
Preconditioner discharge temperature, °C	59.0	82.3	94.1
Extruder shaft speed, rpm	410.2	407.3	378.4
Extruder operational torque, %	65.5	53.6	55.1
Extruder steam flow, kg/h	0.0	0.0	0.0
Extruder water flow, kg/h	1.7	7.8	0.8
Extruder knife speed, rpm	2693.3	3047.0	3041.0
Extruder zone 1 shaft temperature, °C	55.5	54.3	53.4
Extruder zone 2 shaft temperature, °C	78.3	70.3	71.3
Extruder zone 3 shaft temperature, °C	80.7	88.1	91.4
Extruder die pressure, psig	355.0	334.3	328.6
Extruder die temperature, °C	117.7	127.8	114.9

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

Table 3.4: Analyzed chemical composition of extruded feline and canine diets containing select dietary fiber sources at different stages of processing[1].

	Treatments[2]														
	AMD					BPD					CD				
Item[3]	R	P	E	D	CO	R	P	E	D	CO	R	P	E	D	CO
DM, %	92.5	79.3	82.5	96.2	94.3	92.0	79.7	78.2	93.5	93.3	91.9	80.6	80.6	96.1	95.8
	----- DM basis -----														
OM, %	91.5	91.4	91.4	91.9	92.3	91.2	91.4	91.2	92.0	92.7	92.9	92.4	92.3	92.9	93.2
Ash, %	8.5	8.6	8.6	8.1	7.8	8.8	8.6	8.8	8.0	7.3	7.1	7.6	7.7	7.1	6.8
AHF, %	9.4	8.9	9.0	9.8	14.2	5.9	10.0	10.0	8.1	13.0	5.1	10.4	9.7	8.8	15.9
CP, %	36.1	35.4	37.2	38.9	33.9	36.9	37.1	36.8	34.6	31.4	32.9	35.8	35.3	35.6	32.5
TDF, %	21.1	22.4	19.4	19.9	18.5	23.7	20.1	18.7	18.4	19.6	16.3	23.4	17.6	21.0	19.9
SDF, %	3.0	3.3	4.5	5.9	3.1	5.4	2.5	4.7	3.3	3.5	2.6	1.4	0.5	1.9	1.5
IDF, %	18.2	19.1	15.0	14.0	15.4	18.2	17.6	14.0	15.2	16.1	13.7	22.1	17.1	19.0	18.4
GE, kcal/g	4.8	4.8	4.8	4.9	5.1	4.7	4.8	4.8	4.7	5.0	4.7	4.8	4.8	4.8	5.2

[1] R = raw dry ingredients; P = after preconditioner; E = after extruder knife; D = after dryer; CO = after coater.

[2] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

[3] DM = dry matter; OM = organic matter; AHF = acid-hydrolyzed fat; CP = crude protein; TDF = total dietary fiber; SDF = soluble dietary fiber; IDF = insoluble dietary fiber; GE = gross energy.

Table 3.5: Analyzed average physical characteristics of extruded feline and canine diets containing select dietary fiber sources at different stages of processing[1].

	Treatments[2]								
	AMD			BPD			CD		
Item[3]	E	D	CO	E	D	CO	E	D	CO
PM, g	0.14 ± 0.01	0.13 ± 0.02	0.13 ± 0.02	0.14 ± 0.01	0.13 ± 0.01	0.15 ± 0.02	0.12 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
PDi, mm	7.3 ± 0.4	8.4 ± 0.7	8.1 ± 0.6	7.0 ± 0.2	6.6 ± 0.4	6.5 ± 0.5	6.8 ± 0.4	6.3 ± 0.3	6.3 ± 0.3
PL, mm	6.6 ± 0.7	6.3 ± 0.7	6.0 ± 0.3	6.5 ± 0.4	6.5 ± 0.6	6.9 ± 0.6	6.7 ± 0.4	6.3 ± 0.4	6.7 ± 0.7
PV, cm^3	0.27 ± 0.03	0.35 ± 0.09	0.31 ± 0.05	0.25 ± 0.02	0.22 ± 0.04	0.24 ± 0.05	0.24 ± 0.03	0.20 ± 0.03	0.21 ± 0.04
PDe, g/cm^3	0.53 ± 0.05	0.39 ± 0.06	0.42 ± 0.06	0.54 ± 0.04	0.61 ± 0.07	0.66 ± 0.13	0.49 ± 0.05	0.51 ± 0.06	0.57 ± 0.07
SEI	3.3 ± 0.3	4.5 ± 0.8	4.2 ± 0.6	3.1 ± 0.2	2.7 ± 0.3	2.7 ± 0.4	2.9 ± 0.3	2.5 ± 0.3	2.5 ± 0.3
SL, mm/g	4.6 ± 0.3	4.7 ± 0.3	4.7 ± 0.3	4.8 ± 0.2	4.9 ± 0.1	4.6 ± 0.3	5.7 ± 0.2	6.4 ± 0.2	5.7 ± 0.3
KH, N	59.6 ± 10.6	42.2 ± 15.8	44.5 ± 13.4	59.9 ± 4.8	27.4 ± 9.0	33.7 ± 6.1	50.8 ± 10.7	93.0 ± 10.3	85.4 ± 8.6
EC, Nxmm	77.9 ±13.2	39.2 ± 14.4	36.3 ± 5.3	70.2 ± 11.8	16.1 ± 6.6	20.0 ± 6.7	124.1 ± 9.6	89.5 ± 14.8	77.7 ± 12.6

[1] E = after extruder knife; D = after dryer; CO = after coater.

[2] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

[3] PM = piece mass; PDi = piece diameter; PL = piece length; PV = piece volume; PDe = piece density; SEI = sectional expansion index; SL = specific length; KH = kibble hardness; EC = energy to compress kibble 50%.

Figure 3.1: Process flow for manufacturing extruded feline and canine diets containing select dietary fiber sources.

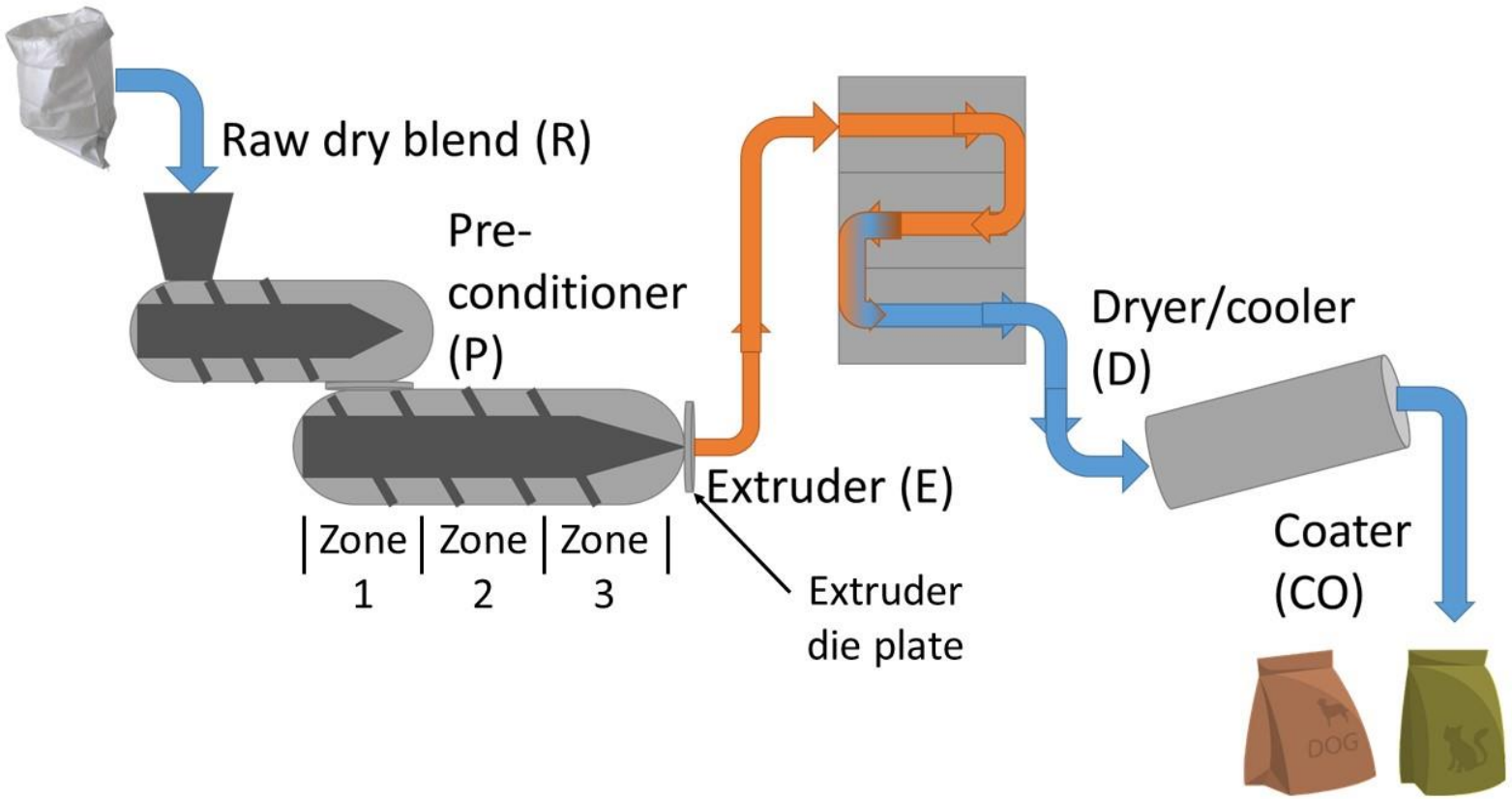
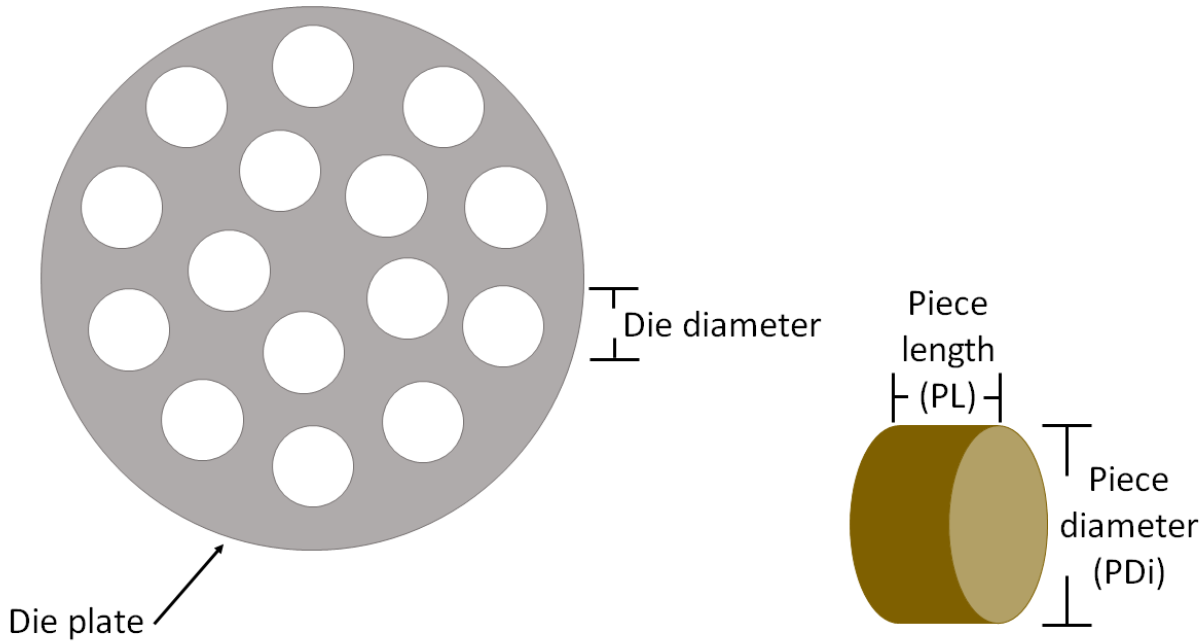


Figure 3.2: Diagram of die diameter, piece length, and piece diameter of extruded feline and canine diets containing select dietary fiber sources.



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CHAPTER 4

THE USE OF AVOCADO MEAL IN AN EXTRUDED DIET FOR ADULT FELINES

ABSTRACT

This experiment tested avocado meal, the coproducts of avocado oil processing, as a novel dietary fiber source in extruded diets for felines. Three diets targeting 15% total dietary fiber (**TDF**) containing either avocado meal (**AMD**), beet pulp (**BPD**), or cellulose (**CD**) were formulated to meet the AAFCO (2016) nutrient profiles for adult cats. Eight neutered male cats [mean age 10.8 ± 0.8 years (**yr**) and mean body weight (**BW**) 5.3 ± 1.7 kilograms (**kg**)] were randomly assigned to the dietary treatments using an incomplete replicated 3x3 Latin square design. Each period lasted 3 weeks with 17 days (**d**) of diet adaption followed by 4 d of fecal and urine collection. Food intake and fecal and urine output were measured and analyzed for apparent total tract digestibility (**ATTD**) calculations. A fresh fecal sample from each animal and period was collected for fermentative end-product analysis. Fecal samples were scored on a 5-point scale (1 = dry, hard feces, 5 = diarrhea). Blood samples were collected at the end of each period for serum chemistry. Data were analyzed using MIXED procedure, SAS, version 9.4. There was no effect ($P > 0.05$) on total or daily fecal output [as-is or dry matter (**DM**) basis], daily DM intake, or ATTD of DM, organic matter, or gross energy. Lower ATTD of crude protein ($P < 0.05$) for AMD (76.6%) was seen in contrast with BPD (82.8%) and CD (86.5%). Acid-hydrolyzed fat ATTD was greater ($P < 0.05$) for CD (93.7%) than AMD (91.4%), with BPD (92.0%) intermediate ($P > 0.05$). Cats fed CD (31.8%) exhibited lower ($P < 0.05$) TDF ATTD than those fed AMD (50.6%) or BPD (52.6%), which did not differ ($P > 0.05$) from each other. Fecal scores for BPD (2.5) and CD (2.2) were different ($P < 0.05$), whereas AMD (2.4)

did not differ (*P > 0.05*) from either. Fecal concentrations of acetate, isobutyrate, and propionate for AMD (309.2, 4.4, and 106.9 μmole/g DM, respectively) were similar (*P > 0.05*) to the BPD treatment (313.3, 3.8, and 84.7 μmole/g DM, respectively). Other fermentative end-products were not affected (*P > 0.05*) by treatment or not present at detectable levels. Serum creatinine was above the reference range for all treatments; however, this colony of cats has a history of high creatinine levels regardless of dietary treatment. In conclusion, avocado meal appears to be an acceptable dietary fiber source for extruded feline diets, resulting in similar nutrient digestibility and fecal quality as dietary fiber sources commonly utilized in commercial pet foods.

INTRODUCTION

Avocado meal is the dried and ground remnants of the avocado fruit after oil has been extracted from the pulp. It can be classified as a fiber source based on its nutrient composition, but contains a moderately low amount of fat. Research supports its use as a fiber source for ruminants (Skenjana et al., 2006) and broiler chickens (van Ryssen et al., 2013), with increasing concentrations causing reductions in performance parameters. Defatted avocado pulp, which does not contain the seed and skin, also resulted in decreased food intake and BW gain at higher inclusion levels in rats (Naveh et al., 2002). Unfortunately, no study using felines has been published and no previous study has addressed the impact avocado meal may have on gastrointestinal fermentation.

The objective of this study was to assess the use of avocado meal as a dietary fiber source in a diet for adult cats. Diets containing either beet pulp or cellulose were used as comparisons, since both are standard fiber sources in the pet food industry. We hypothesized that avocado

meal would have an intermediate fermentative profile when compared with beet pulp and cellulose due to its moderate fiber content and ratio of insoluble to soluble dietary fiber. We also expected that avocado meal would not negatively impact apparent total tract digestibility of nutrients and would promote gastrointestinal fermentation by microbiota as measured as fecal concentrations of fermentative end-products.

MATERIALS AND METHODS

Animals

All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. Eight neutered male cats (10.8 ± 0.8 yr, 5.31 ± 1.70 kg, and 5.13 ± 0.40 body condition score, **BCS**) were used in an incomplete replicated 3x3 Latin square design. Each period lasted for 21 d, with 17 d of adaptation and 4 d of total fecal and urine collection. Cats were fed individually at 0800 and 1500 and had access to food for 2 h. Otherwise, cats were housed in a group during the adaptation phase and had free access to water. During the collection phase, cats were housed individually and were fed at the same times with free access to water.

Diets

Three diets were formulated to meet the AAFCO (2016) nutrient profiles for adult cats with similar macronutrient composition (**Table 4.1**). Each diet contained either avocado meal (**AMD**), beet pulp (**BPD**), or cellulose (**CD**) as the main total dietary fiber sources. The avocado meal ingredient was sourced from Green Source Organics Natural Extracts (Boynton Beach, FL) and all other dry ingredients were procured from Lortscher Animal Nutrition (Bern, KS). Diets were manufactured at the Kansas State University Bioprocessing and Industrial Value Added Products Center (Manhattan, KS) and coated with an additional 4% choice white grease

(University of Illinois Feed Mill, -Champaign, IL) and 2% palatant (AFB International bioflavor #F25003, St. Charles, MO). Cats were fed to maintain BW and BCS, which were measured once a week throughout the experimental periods.

Acceptability Testing

The AMD without the second coating of choice white grease and palatant was tested for acceptability by Kennelwood, Inc. (Champaign, IL) using a monadic test. Twenty cats (4.58 ± 1.09 kg) were offered 100 g of the diet each day for 2 d. Refusals were recorded and daily consumption calculated.

Sample Collection

During the collection phase, all feces were collected from each cat for macronutrient analysis. Fecal weight (as-is basis) and fecal score using a 5-point scale (1 = dry, hard pellets; 5 = diarrhea) were recorded for each sample. Samples were frozen at -20 ºC for analysis at a later time. Total urine output volume also was recorded and approximately 25% of each sample was saved for further analysis. Urine was immediately acidified with 10 milliliters (**mL**) of 2N hydrochloric acid during the collection periods. Samples from each cat were stored in separate containers and frozen at -20 ℃.

One fecal sample from each cat for each period was collected within 15 minutes (**min**) of defecation. Fecal score and total sample weight were recorded. pH was measured using a Denver Instrument AP10 pH meter (Denver Instrument, Bohemia, NY) with a Beckman electrode (Beckman Instruments, Inc., Fullerton, CA). Absolute DM concentration of each sample was determined by drying approximately 2 g in duplicate in a 105 ℃ oven until all moisture was removed. Two grams (**g**) in duplicate were frozen at – 20 ℃ for phenol and indole analyses. Five g of feces were stored, mixed with five mL of 2N hydrochloric acid, and frozen at -20 ℃ for

analysis of ammonia, short-chain fatty acids (**SCFA**), and branched-chain fatty acids (**BCFA**). Any remaining sample was frozen at -20 °C for macronutrient analyses.

A venous blood sample (6 mL) was collected from each cat at the beginning of each collection period. One milliliter was placed in EDTA vacutainer tubes (BD Vacutainer©) for a complete blood count and put on ice for approximately 30 min. The remaining 5 mL were placed in serum vacutainer tubes (BD Vacutainer©) for serum chemistry profile, stored at room temperature for 30 min, and centrifuged at 1240 g for 10 min at 4 °C. All analyses were done by the Clinical Pathology group at the University of Illinois Veterinary School (Urbana, IL).

Chemical Analyses

Diets were subsampled and ground in a Wiley mill with a 10 mesh (2 millimeter, **mm**) screen size. Feces from each cat and period were pooled together and dried in a 55 °C forced-air oven before grinding in a model 4 Wiley Mill (Thomas Scientific, Swedesboro, NJ) with a 10 mesh (2 mm) screen size. Each analysis was done in duplicate and results were deemed successful if error between duplicates was less than 5%. Dry matter (**DM**) and organic matter (**OM**) were determined following the AOAC 2006 procedure (methods 934.01 and 942.05, respectively). Acid-hydrolyzed fat (**AHF**) of the diet and feces was done following methods from the American Association of Cereal Chemists (Method 30-14) and Budde et al. (1952). Total crude protein (**CP**) analysis was done by measuring total nitrogen using a LECO TruMac (model 630-300-300, St. Joseph, MI) and following AOAC Method 992.15 (2006). Gross energy was measured using a Parr 6200 calorimeter (Parr Instrument Company, Moline, IL). Total dietary fiber (TDF) was measured by Eurofins (Des Moines, IA) following AOAC 991.43.

Volatile fatty acids (SCFA and BCFA) were analyzed using gas chromatography with a glass 6'x1/4" ODx4mmID column and 10%SP1200/1%H_3PO_4 on 80/100 Chrom-WAW, Supleco

packing and following the methods of Erwin et al. (1961), Supleco Inc. (1975), and Goodall and Byers (1978). Gas chromatography also was used to measure phenols and indoles according to methods described by Flickinger et al. (2003). Ammonia concentration was determined using the methods of Chaney and Marbach (1962).

Physical Analyses

Ten kibbles for AMD, BPD, and CD were weighed individually on an analytical scale (model #AG104, Mettler Toledo, Switzerland). Digital calipers (model #01407A, Neiko Tools, China) were used to determine radial diameter and piece length. From these measurements, piece volume, piece density, sectional expansion index (**SEI**), and specific length were calculated using **Equations 4.1-4.4**. Duplications for each diet were averaged and sample standard deviation was calculated.

Equation 4.1: Piece volume formula

$$Piece\ volume\ (cm^3) = \pi * (Piece\ diameter\ in\ cm)^2 * \frac{(Piece\ length\ in\ cm)}{4}$$

Equation 4.2: Piece density formula

$$Piece\ density\ \left({}^{g}/_{cm^3}\right) = \frac{Piece\ mass\ in\ g}{Piece\ volume\ in\ cm^3}$$

Equation 4.3: Sectional expansion index (SEI) formula

$$SEI = \frac{(Piece\ diameter\ in\ mm)^2}{(Die\ diameter\ in\ mm)^2}$$

Equation 4.4: Specific length formula

$$Specific\ length\ \left({}^{mm}/_{g}\right) = \frac{Piece\ length\ in\ mm}{Piece\ mass\ in\ g}$$

A texture analyzer (TA HD *plus*; Texture Technologies Corporation, Scarsdale, NY) equipped with a 30 kg load cell and a 50.8 mm cylindrical probe (TA-15) was used to compress 10 kibbles per diet by 50%. Pre-test, test, and post-test speed settings were 2, 1, and 10 mm/s, respectively. Data was collected using Texture Expert Exceed software (Stable Micro Systems, Godalming, England). Peak force [greatest force measurement; Newtons (**N**)] and energy required for compression [calculated from area under the curve; Newton x millimeter (**Nxmm**)] were calculated from time vs. force graphs. This method was slightly modified from Dogan et al. (2007). Duplicates for each diet and processing stage combination were averaged together and standard deviation was calculated.

Calculations

Digestibility of individual macronutrients was calculated by subtracting the nutrient content of the feces from the nutrient content of the diet consumed, dividing by the nutrient content of the diet, and multiplying by 100. Nutrient content of feces and diet were determined by multiplying the feces or food intake (g, DM basis) by the corresponding nutrient percentage. Digestible energy was calculated by subtracting the energy contained in the feces from the energy contained in the diet. Metabolizable energy was determined by subtracting the energy contained in the urine from the digestible energy.

Statistical Analysis

Data from these calculations were compared using SAS, version 9.4 using a mixed model. The statistical model included the fixed effect of diet and the random effect of animal. Data normality was checked using the UNIVARIATE procedure of SAS. All treatment least-square means were compared with each other and Tukey adjustment was used to control for

experiment-wise error. P-values less than 0.05 were considered statistically different, whereas p-values greater than 0.05 and less than 0.10 were considered a trend.

RESULTS

Ingredient formulations for AMD, BPD, and CD were kept similar, with avocado meal or beet pulp replacing cellulose in their respective formulations (**Table 4.1**). Chicken by-product meal and rice concentrations were adjusted in AMD and BPD to obtain similar nutrient concentrations (**Table 4.2**). The CD contained more AHF and TDF than AMD and BPD.

Descriptive physical characteristics of the dietary treatments are shown in **Table 4.3**. Mean piece mass (0.13 - 0.15 g) and piece length (6.2 - 6.9 mm) were similar for all three diets. The AMD (8.5 ± 0.4 mm) had greater expansion than the BPD (7.0 ± 0.3 mm) and CD (6.1 ± 0.3 mm). This same relationship was observed with piece volume (AMD = 0.35 ± 0.04; BPD = 0.25 ± 0.03; CD = 0.20 ± 0.03) and SEI (AMD = 4.5 ± 0.4; BPD = 3.1 ± 0.3; CD = 2.3 ± 0.2). Piece density for BPD (0.61 g/cm^3) and CD (0.64 ± g/cm^3) were greater than AMD (0.40 ± 0.04 g/cm^3). The CD (5.5 ± 0.5 mm/g) also had longer specific length than AMD (4.5 ± 0.3 mm/g) and BPD (4.3 ± 0.2 mm/g), which were very similar to each other. The AMD and BPD exhibited very similar kibble hardness (34.4 ± 6.4 N and 38.7 ± 7.0 N, respectively) and energy to compress (35.1 ± 11.0 Nxmm and 24.1 ± 5.8 Nxmm, respectively), while CD (84.3 ± 19.6 N and 101.5 Nxmm, respectively) was greater than both.

Daily DM intake was not affected ($P > 0.05$) by the dietary treatments (**Table 4.4**). Organic matter daily intake, on a DM basis, trended ($P < 0.10$) to be greatest in cats fed CD (70.1 g/d) and lowest for AMD (61.2 g/d), with BPD (96.6 g/d) being intermediate ($P > 0.10$). Both daily intake of AHF and CP were lowest ($P < 0.05$) for cats fed AMD (11.2 g/d and 21.4

g/d, respectively), with CD (14.9 g/d and 24.5 g/d, respectively) and BPD (13.5 g/d and 26.4 g/d, respectively) being similar ($P > 0.05$) to each other. Cats consuming CD (14.6 g/d) ate the most ($P < 0.05$) TDF, followed by BPD (12.1 g/d; $P < 0.05$), then AMD (11.4 g/d; $P < 0.05$). BPD cats (4.0 g/d) consumed the most ($P < 0.05$) soluble dietary fiber, with AMD (3.1 g/d) consuming less ($P < 0.05$), and CD (1.3 g/d) consuming the least ($P < 0.05$). Cats fed CD (10.2 g/d) consumed the most ($P < 0.05$) insoluble dietary fiber with BPD (5.7 g/d) and AMD (4.9 g/d) not being different ($P > 0.05$) from each other. AMD-fed cats (349.0 kcal/d) tended ($P < 0.10$) to consume the least gross energy (**GE**) compared to BPD (401.2 kcal/d) and CD (405.1 kcal/d), which were the same ($P > 0.10$). Daily fecal output on an as-is and DM basis were not different ($P < 0.05$) among cats fed any of the diets. Fecal score was highest ($P < 0.05$) for BPD (2.5), CD (2.1) the lowest ($P < 0.05$), and AMD (2.4) being similar to BPD and CD ($P > 0.05$). The CD-fed cats (46.2%) had greater ($P < 0.05$) fecal DM content than BPD (34.0%) and AMD (33.5%), which were not different from each other ($P > 0.05$). Fecal pH was not impacted ($P > 0.05$) by the dietary treatments.

Apparent total tract DM and OM digestibilities were not affected ($P > 0.05$) by treatment (**Table 4.4**). Acid hydrolyzed fat ATTD was greatest ($P < 0.05$) for cats fed CD (93.7%, DM basis), lowest for AMD (91.4%, DM basis; $P < 0.05$), and BPD (92.0%, DM basis) was not different ($P > 0.05$) from either CD or AMD. The AMD-fed cats (76.6%, DM basis) exhibited lower ($P < 0.05$) CP ATTD than BPD- (82.8%, DM basis) and CD-fed cats (86.5%, DM basis), which were not different ($P > 0.05$) from each other. Cats consuming AMD (50.6%, DM basis) and BPD (52.6%, DM basis) exhibited greater ($P < 0.05$) TDF ATTD than CD (31.8%, DM basis), but were similar ($P > 0.05$) to each other. While ATTD of GE was not impacted ($P > 0.05$) by treatments, energy partitioning was. Digestible energy tended to be lower ($P < 0.10$) for

AMD (4.2 kcal/g, DM basis) than BPD (4.4 kcal/g, DM basis) and CD (4.4 kcal/g, DM basis), which were not different from each other ($P > 0.10$). Similarly, metabolizable energy of CD (4.2 kcal/g, DM basis) was greater ($P < 0.05$) than AMD (3.9 kcal/g, DM basis) and BPD (4.1 kcal/g, DM basis) was not different ($P > 0.05$) from either.

Fecal concentrations of total SCFA and acetate were significantly lower ($P < 0.05$) for cats fed CD (249.2 μmole/g DM and 153.7 μmole/g DM, respectively), and in both instances, AMD- (483.9 μmole/g DM and 309.2 μmole/g DM, respectively) and BPD-fed cats (456.6 μmole/g DM and 313.3 μmole/g DM, respectively) were not different ($P > 0.05$) from each other (**Table 4.5**). Butyrate concentration was not affected by dietary treatment ($P > 0.05$). Fecal concentration of propionate was greater for cats fed AMD (106.9 μmole/g DM; $P < 0.05$) than CD (56.2 μmole/g DM), and intermediate ($P > 0.05$) for BPD (84.7 μmole/g DM). The same relationship was observed for total BCFA concentration (AMD = 14.7 μmole/g DM; BPD = 13.9 μmole/g DM; CD = 10.1 μmole/g DM). Isobutyrate concentration for AMD-fed cats (4.4 μmole/g DM) and BPD (3.8 μmole/g DM) were greater ($P < 0.05$) than those consuming CD (2.4 μmole/g DM), but were not different from each other ($P > 0.05$). Isovalerate, valerate, and ammonia concentrations did not differ among dietary treatments ($P > 0.05$). Fecal indole and total indole and phenol concentrations trended to be greatest ($P < 0.10$) when cats were fed AMD (7.7 μmole/g DM and 10.1 μmole/g DM, respectively), but similar for BPD (4.2 μmole/g DM and 5.2 μmole/g DM, respectively) and CD (3.9 μmole/g DM and 5.5 μmole/g DM; $P > 0.10$). Concentration of 4-methyl-phenol was not affected ($P > 0.05$), and all additional individual phenol and indole compounds were not present at detectable levels.

Most serum metabolites were not affected ($P > 0.05$) by the dietary treatments and were within reference ranges for healthy adult cats provided by the University of Illinois Veterinary

Diagnostic Laboratory for adult felines (**Table 4.6**). Serum creatinine levels were above reference values for all dietary treatments. However, it tended to be greater ($P < 0.10$) for AMD-fed cats (1.8 mg/dL) in contrast to CD (1.7 mg/dL) while BPD (1.7 mg/dL) did not differ ($P > 0.10$) from either. The ratio of sodium to potassium for cats consuming CD (27.9) was lower than the reference range, but was not different ($P > 0.05$) from BPD (32.6) and AMD (33.3), which were within the reference range. Alkaline phosphate total concentrations were within reference range, but BPD- (24.9 U/L) and CD-fed cats (24.2 U/L) were lower ($P < 0.05$) than AMD-fed cats (35.7 U/L). Alanine aminotransferase levels for cats fed AMD (57.9 U/L) were greater ($P < 0.05$) than those fed BPD (47.0 U/L) and CD (51.7 U/L) was not different from either ($P > 0.05$). Finally, cholesterol levels (AMD = 171.2 mg/dL; BPD = 164.1 mg/dL; CD = 169.1 mg/dL) were not affected ($P > 0.05$) by treatment, but all were higher than the acceptable upper limit.

Complete blood counts for cats fed all three dietary treatments were within normal ranges (**Table 4.7**). Mean cell volume tended to be higher ($P < 0.10$) for cats fed BPD (43.5 fl) than cats fed AMD (43.1 fl) or CD (43.1 fl), which were not different ($P > 0.10$) from each other. The AMD treatment (14.2 pg) exhibited lower ($P < 0.05$) mean corpuscular hemoglobin when fed to cats, with BPD (14.3 pg) and CD (14.3 pg) being similar ($P > 0.05$). All other measurements were not different ($P > 0.05$).

The monadic diet acceptability test resulted in an average daily consumption of 30.6 ± 32.7 g and 27.3 ± 30.4 g, and total consumption for both days was 57.8 ± 61.7 in a 2-d period (**Figure 4.1**).

DISCUSSION

A recent increase in the interest in pet nutrition and gut health has driven the need for more research on dietary fiber sources for cats, where previously fiber was not considered a major concern for carnivorous felines. Today, we know that dietary fibers can decrease caloric density of pet foods, aid in weight loss programs, and serve as nutrients for microbes in the gastro-intestinal tract. There is a growing need for new fiber sources and there is limited information about the effects of extruding high fiber diets for cats. As such, this study aimed to: 1) describe the effects of different fiber sources on the physico-chemical characteristics of the diets tested herein, and 2) compare AMD to BPD and CD in terms of macronutrient ATTD and concentrations of fermentative end-products.

The three dietary treatments differed in some physical and organoleptic characteristics, but this was expected due to the differences in SDF and IDF concentrations. Yanniotis et al. (2007) found that extrudates with IDF supplementation were less expanded and porous and harder than extrudates with SDF supplementation. In this experiment, AMD and BPD had higher SDF than CD and, as such, these treatments were more expanded, with higher piece volume and SEI, less hard, and required less energy to compress. However, this study does not suggest a preference for CD vs. AMD or BPD, as similar intakes were observed during the digestibility experiment.

The BPD and CD acted as good comparisons in this study and agreed with previously published literature. Bueno et al. (2000) evaluated the effects of different fiber sources on concentrations of SCFA and BCFA. They found that beet pulp resulted in greater absorption of acetate compared to cellulose when given a perfusion of SCFA, which suggests that cats fed beet pulp are better adapted to absorb acetate than those fed cellulose because beet pulp is more

fermentable and fermentation yields more acetate than cellulose. This agrees with the present study with greater acetate concentration found in cats fed BPD compared to CD. Those authors also reported no significant difference with total SCFA and propionate (although, numerically, concentration was greater for beet pulp), but greater butyrate concentrations with beet pulp than cellulose. Our study also saw no significant difference in total SCFA, but significance for propionate but not butyrate. The differences in significance could be caused by differences in TDF; Bueno et al. (2000) reported values of 8.4% and 8.8%, whereas the present study was 12.7 and 15.1%.

A few differences were between the present study and previous studies assessing avocado meal or defatted avocado pulp. Naveh et al. (2002) showed that feeding avocado meal to rats decreased food intake and fecal output when compared to rats fed cellulose, and van Ryssen at al. (2013) observed that avocado meal decreased voluntary food intake with increasing inclusion levels when fed to broiler chickens. Our study utilized adult felines, which have different digestive physiology than rats and broiler chickens, and controlled for BW gain by adjusting the amount of food offered. This could have caused the different results observed in our study.

In this study, AMD performed most similarly to BPD than to CD. The AMD and BPD were very close in SDF and IDF percentages and were drastically different from CD. This means that AMD and BPD have comparable fermentabilities and are more fermentable than CD. This is similar to the relationship reported by Barry et al. (2010), where diets containing fructooligosaccharides and pectin had a similar IDF:SDF ratio and performed similarly, even though there were differences in diet percentages. Both the present study and Barry et al. (2010) showed no significant difference in fecal score or pH between the previously mentioned treatments and showed similar relationships with SCFA, BCFA, ammonia, 4-methyl phenol, and

indole. This suggests that even though TDF concentrations in our study are not the same, the IDF:SDF ratio may have a bigger impact.

Fischer et al. (2012) showed that more fermentable diets (i.e., 15.5% beet pulp, as-is basis) cause lower fecal DM than less fermentable diets (i.e., 24.9% wheat bran, as-is basis), which is also supported by this study. They also reported significantly lower ATTD of DM, OM, and energy and significantly higher ATTD of TDF when cats were fed the more fermentable diet. This study did not report statistical difference between the treatments for DM, OM, or energy digestibility. The trending differences in intake of OM and energy could have confounded the effect of the treatments on those ATTDs. We saw similar metabolizable energy values between AMD and BPD. The CD exhibited lower TDF ATTD than either AMD or CD, which agrees with Fischer et al. (2012) and supports the idea that avocado meal and beet pulp have similar fermentability in cats. As such, we would expect to see AMD and BPD produce similar concentrations of fermentation end-products.

In general, the cats in our study remained healthy. All blood cell measurements were within normal ranges for healthy adult cats. Only two serum metabolites, creatinine and total cholesterol, were above references ranges for healthy adult felines. Higher creatinine levels suggest kidney disease and are common in older cats, such as those used in this study. Cats fed the AMD tended to have higher creatinine levels than those fed the BPD and CD, but the 0.1 mg/dL difference would not likely cause clinical symptoms. This cat colony is regularly monitored by veterinarians, and all animals were considered to be healthy prior, during, and after the completion of this study. In addition, senior-aged cats (as were the cats used in this study) have an acceptable range in serum creatinine levels from 1.6 to 2.1 mg/dL (American Association of Feline Practitioners and Academy of Feline Medicine, 2005). The average

concentration for cats fed AMD falls within this range, so we do not believe serum creatinine was an issue for these felines. Total cholesterol was not significantly different across treatments, although cats fed the AMD had the highest numerical values. Urine of AMD-fed cats exhibited a red color and BPD-fed cats had visibly darker urine than CD-fed cats. Anthocyanins, which are found in the skin of the avocado (Ashton et al., 2006) and in sugar beets, are water-soluble pigments and would be concentrated in the processing of avocado meal and beet pulp. It is possible that the discoloration was caused by these compounds.

Research on persin and avocado toxicity in cats has not been published. In this study, no blood cells were outside normal ranges for adult felines. Grant et al. (1991) found elevated levels of blood urea nitrogen in a case study with sheep, but the cats in this study did not show treatment effects and levels for all treatments were within the acceptable range. The only case study published in dogs showed elevated levels of alanine aminotransferase and alkaline phosphatase (Buoro et al., 1994). Cats in the present study had normal levels for both serum metabolites in all three treatments. Alanine aminotransferase was significantly higher in AMD than BPD and CD and alkaline phosphatase was higher in AMD than BPD. Ali et al. (2010) reported high levels of sodium and low levels of chloride and phosphorus in rabbits fed avocado leaves. Treatment effects on sodium, chloride, and phosphorus were not observed, and all treatments were within acceptable ranges. High levels of lymphocytes and moderate levels of neutrophils were reported by Buoro et al. (1994), but were not observed in this study.

Monadic testing of AMD at lower fat concentration (~14%) yielded less than desirable average daily food intake (~30 g/d). This amount may not provide sufficient nutrients over an extended period of time. The Monadic test also revealed a high standard deviation, which suggests that consumption varies with each cat's personal preferences. An additional 4% choice

white grease and 2% palatant was added to the kibble as a coating and this resulted in an increase in average daily food intake (~70 g/d). As such, we believe that adding highly appealing ingredients to the outside of the kibble may increase acceptability of a high fiber diet containing avocado meal.

CONCLUSION

The present study showed that avocado meal can be an acceptable dietary fiber source for adult felines. It performs similarly to beet pulp in terms of ATTD and gastrointestinal fermentation. Serum chemistry and complete blood cell analyses showed no detrimental effects on health or gastrointestinal tolerance to the diets. This study utilized diets with higher TDF concentrations than what is found in most commercial products. It is possible that a diet containing less avocado meal will have improved diet acceptability and palatability, without requiring additional kibble coating. Overall, avocado meal appears to be a safe fiber source in feline diets; however, future studies should verify that no adverse effects are observed in cats fed avocado-derived ingredients over longer periods of time (i.e., months or years).

TABLES AND FIGURE

Table 4.1: Ingredient composition of dietary treatments containing select dietary fiber sources for adult felines.

	Treatments[1]		
Ingredient, % as-is	AMD	BPD	CD
Chicken by-product meal	29.57	29.91	31.42
Brewer's rice	26.81	27.17	31.61
Choice white grease	9.11	11.32	11.32
Corn gluten meal	7.66	7.74	7.74
Whole corn	3.83	3.87	3.87
AFB bioflavor F25003	1.89	1.89	1.89
Avocado meal	18.67	0.00	0.00
Beet pulp	0.00	15.66	0.00
Cellulose	0.00	0.00	9.72
AFB bioflavor B22006	0.89	0.89	0.89
Salt	0.48	0.47	0.47
Potassium chloride	0.43	0.42	0.42
Taurine	0.19	0.19	0.19
Mineral premix[2]	0.17	0.17	0.17
Vitamin premix[3]	0.17	0.17	0.17
Choline chloride	0.12	0.12	0.12

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

[2] Provided per kilogram of diet: 35.6 mg manganese ($MnSO_4$), 601.4 milligrams iron ($FeSO_4$), 29.6 mg copper ($CuSO_4$), 0.02 mg cobalt ($CoSO_4$), 326.3 mg zinc ($ZnSO_4$), 2.9 mg iodine (KI), and 0.8 mg selenium (Na_2SeO_3).

[3] Provided per kilogram of diet: 17000 IU vitamin A (retinyl acetate), 2550 IU vitamin D3, 136 IU vitamin E (DL-α tocopherol acetate), 3.3 mg vitamin K, 28.9 mg thiamin, 28.9 mg riboflavin, 51.7 mg pantothenic acid, 117.3 mg niacin, 28.9 mg pyridoxine, 0.1 mg biotin, 1.0 mg folic acid, and 1.1 mg vitamin B12 (mannitol).

Table 4.2: Analyzed chemical composition of dietary treatments containing select dietary fiber sources for adult felines.

	Treatments[1]		
Item	AMD	BPD	CD
Dry matter, %	94.9	93.0	94.4
		----- % DM basis -----	
Organic matter	91.4	91.3	92.8
Ash	8.6	8.7	7.2
Acid hydrolyzed fat	16.7	17.7	19.7
Crude protein	31.9	34.7	32.1
Total dietary fiber	11.9	12.7	15.1
Soluble dietary fiber	4.6	5.2	1.7
Insoluble dietary fiber	7.3	7.5	13.5
Gross energy, kcal/g	5.2	5.3	5.4

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

Table 4.3: Analyzed physical characteristics of extruded dietary treatments for adult felines containing select dietary fiber sources.

	Treatments[1] (Average ± Standard Deviation)		
Item	AMD	BPD	CD
Piece mass, g	0.14 ± 0.01	0.15 ± 0.01	0.13 ± 0.01
Piece diameter, mm	8.5 ± 0.4	7.0 ± 0.3	6.1 ± 0.3
Piece length, mm	6.2 ± 0.3	6.4 ± 0.5	6.9 ± 0.4
Piece volume, cm^3	0.35 ± 0.04	0.25 ± 0.03	0.20 ± 0.03
Piece density, g/cm^3	0.40 ± 0.04	0.61 ± 0.07	0.64 ± 0.09
Sectional expansion index	4.5 ± 0.4	3.1 ± 0.3	2.3 ± 0.2
Specific length, mm/g	4.5 ± 0.3	4.3 ± 0.2	5.5 ± 0.5
Kibble hardness, N	34.4 ± 6.4	38.7 ± 7.0	84.3 ± 19.6
Energy to compress 50%, Nxmm	35.1 ± 11.0	24.1 ± 5.8	101.5 ± 19.5

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

Table 4.4: Food intake, fecal characteristics, and total tract apparent macronutrient digestibility by adult felines fed dietary treatments containing select dietary fiber sources.

	Treatments[1]			
Item	AMD	BPD	CD	SEM[†]
Food intake, as-is				
Dry matter, g/d	67.9	71.6	73.6	4.23
Organic matter, g/d	61.2[y]	69.6[xy]	70.1[x]	2.74
Acid hydrolyzed fat, g/d	11.2[b]	13.5[a]	14.9[a]	0.54
Crude protein, g/d	21.4[b]	26.4[a]	24.5[a]	0.96
Total dietary fiber, g/d	11.4[c]	12.1[b]	14.6[a]	0.01
Soluble dietary fiber, g/d	3.1[b]	4.0[a]	1.3[c]	0.11
Insoluble dietary fiber, g/d	4.9[b]	5.7[b]	10.2[a]	0.31
Gross energy, kcal/d	349.0[y]	401.2[x]	405.1[x]	15.71
Fecal output, g/d (as-is)	46.5	51.9	39.5	4.28
Fecal output, g/d (DM basis)	16.1	16.8	18.2	0.62
Fecal score	2.4[ab]	2.5[a]	2.2[b]	0.01
Fecal dry matter, %	33.5[b]	34.0[b]	46.2[a]	2.13
Fecal pH	5.6	6.2	6.0	0.26
Digestibility, %				
Dry matter	76.0	77.9	76.3	1.64
		-----% DM basis -----		
Organic matter	79.4	82.2	79.4	1.39
Acid hydrolyzed fat	91.4[b]	92.0[ab]	93.7[a]	0.72
Crude protein	76.6[b]	82.8[a]	86.5[a]	1.57
Total dietary fiber	50.6[a]	52.6[a]	31.8[b]	4.77
Energy	80.7	83.9	82.8	1.25
Digestible energy, kcal/g	4.2[y]	4.4[x]	4.4[x]	0.07
Metabolizable energy, kcal/g	3.9[b]	4.1[ab]	4.2[a]	0.07

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

[a-c] Superscripts with different letters in a row represent statistical differences ($P < 0.05$).

[x-y] Superscripts with different letters in a row represent trending differences ($0.05 < P < 0.10$).

[†] Pooled standard error of means.

Table 4.5: Fecal fermentative end products for adult felines fed dietary treatments containing select dietary fiber sources.

	Treatments[1]			
Item, μmole/g DM	AMD	BPD	CD	SEM[†]
Total short-chain fatty acids	483.9[a]	456.6[a]	249.2[b]	58.09
Acetate	309.2[a]	313.3[a]	153.7[b]	40.45
Butyrate	67.8	58.9	39.6	10.05
Propionate	106.9[a]	84.7[ab]	56.2[b]	13.46
Total branched-chain fatty acids	14.7[a]	13.9[ab]	10.1[b]	1.85
Isobutyrate	4.4[a]	3.8[a]	2.4[b]	0.62
Isovalerate	6.6	6.1	4.5	0.21
Valerate	3.6	4.4	3.5	0.64
Ammonia	120.9	92.2	109.9	11.17
Total indoles and phenols	10.1[x]	5.2[y]	5.5[y]	1.46
Indole	7.7[x]	4.2[y]	3.9[y]	1.07
4-methyl phenol	2.4	0.7	1.6	0.61

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet

[a-b] Superscripts with different letters in a row represent statistical differences ($P < 0.05$).

[x-y] Superscripts with different letters in a row represent trending differences ($0.05 < P < 0.10$).

[†] Pooled standard error of means.

Table 4.6: Fasted serum chemistry profiles of adult felines fed dietary treatments containing select dietary fiber sources.

		Treatments[1]			
Item	Reference Range	AMD	BPD	CD	SEM[†]
Creatinine, mg/dL	0.4 - 1.6	1.8[x]	1.7[xy]	1.7[y]	0.09
Blood urea nitrogen, mg/dL	18 - 38	27.6	27.1	25.9	0.86
Total protein, g/dL	5.8 - 8.0	6.6	6.6	6.8	0.32
Albumin, g/dL	2.8 - 4.1	3.2	3.2	3.2	0.07
Globulin, g/dL	2.6 - 5.1	3.4	3.5	3.6	0.28
Albumin/Globulin ratio	0.6 - 1.1	1.0	1.0	0.9	0.09
Calcium, mg/dL	8.8 - 10.2	9.6	9.5	9.4	0.16
Phosphorus, mg/dL	3.2 - 5.3	4.2	4.7	4.4	0.17
Sodium, mmol/L	145 - 157	148.3	149.1	149.1	0.63
Potassium, mmol/L	3.6 - 5.3	4.5	4.6	4.7	0.10
Sodium/Potassium ratio	28 - 36	33.3	32.6	27.9	2.39
Chloride, mmol/L	109 - 126	125.7	115.5	115.5	0.76
Glucose, mg/dL	60 - 122	83.0	82.9	79.7	4.90
Alkaline phosphatase total, U/L	10 - 85	35.7[a]	24.9[b]	24.2[b]	2.21
Alanine aminotransferase, U/L	14 - 71	57.9[a]	47.0[b]	51.7[ab]	3.32
Gamma-glutamyl transferase, U/L	0 - 3	0.6	0.4	0.4	0.20
Total bilirubin, mg/dL	0.0 - 0.3	0.1	0.1	0.1	0.02
Cholesterol total, mg/dL	66 - 160	171.2	164.1	169.1	10.49
Triglycerides, mg/dL	21 - 166	33.8	39.2	40.9	3.70
Bicarbonate (TCO_2), mmol/L	12 - 21	17.8	17.5	17.5	0.46
Anion gap	10 - 27	19.6	20.8	20.5	0.98

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

[a-b] Superscripts with different letters in a row represent statistical differences ($P < 0.05$).

[x-y] Superscripts with different letters in a row represent statistical differences ($0.05 < P < 0.10$).

[†] Pooled standard error of means.

Table 4.7: Fasted complete blood cell analysis of adult felines fed dietary treatments with select dietary fiber sources.

Item	Reference range	Treatments[1]			SEM[†]
		AMD	BPD	CD	
Red blood cells, $x10^6/\mu L$	5.00-10.00	8.8	9.2	8.9	0.30
Hemoglobin, g/dL	8.0-15.0	12.4	12.8	12.7	0.43
Hematocrit, %	30.0-45.0	37.9	38.7	38.0	1.24
Mean cell volume, fl	39.0-55.0	43.1[y]	43.5[x]	43.1[y]	1.45
Mean corpuscular hemoglobin, pg	13.0-18.0	14.2[b]	14.3[a]	14.3[a]	0.48
Mean corpuscular hemoglobin concentration, g/dL	30.0-36.0	32.9	32.9	33.3	0.21
Platelet estimate, $x10^3/\mu L$	300-700	317.6	380.4	396.1	38.97
White blood cell count, $x10^3/\mu L$	5.50-19.50	9.8	9.1	9.8	0.93
Sequential neutrophils, $x10^3/\mu L$	2.50-12.50	6.0	5.2	6.4	0.76
Band neutrophils, $x10^3/\mu L$	0.00-0.30	0.0	0.0	0.0	0.00
Lymphocytes, $x10^3/\mu L$	1.00-7.00	2.7	3.3	3.1	0.76
Monocytes, $x10^3/\mu L$	0.00-0.90	0.2	0.2	0.1	0.07
Eosinophils, $x10^3/\mu L$	0.00-0.80	0.6	0.6	0.7	0.18
Basophils, $x10^3/\mu L$	0.00-2.00	0.0	0.0	0.0	0.00

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

[a-b] Superscripts with different letters in a row represent statistical differences ($P < 0.05$).

[x-y] Superscripts with different letters in a row represent trending differences ($0.05 < P < 0.10$).

[†] Pooled standard error of means.

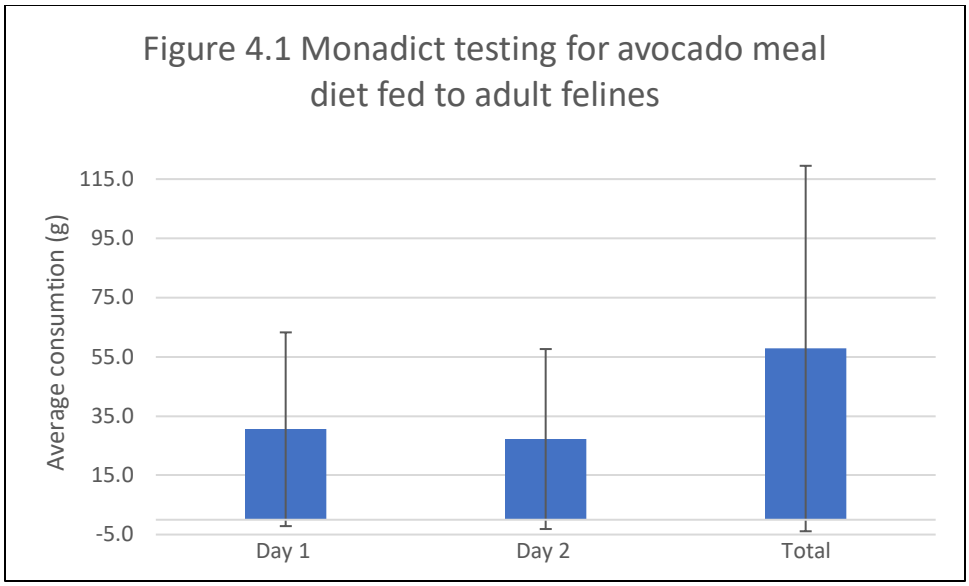
Figure 4.1 Monadict testing for avocado meal diet fed to adult felines
Average consumtion (g)
115.0
95.0
75.0
55.0
35.0
15.0
-5.0
Day 1
Day 2
Total

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CHAPTER 5

THE USE OF AVOCADO MEAL IN AN EXTRUDED DIET FOR ADULT CANINES

ABSTRACT

This study assessed the effects of a diet containing avocado meal (**AMD**), an underutilized by-product avocado oil processing, on apparent total tract digestibility (**ATTD**) and fecal fermentative end-products when compared to beet pulp (**BPD**) and cellulose (**CD**) diets targeting 15% total dietary fiber (**TDF**). Nine intact female beagles (4.9 ± 0.6 yr and 11.98 ± 1.76 kg) were randomly grouped for a 3 x 3 replicated Latin square design. Periods were 14 d long, with 10 d of adaptation followed by 4 d of total fecal and urine collection for apparent total tract digestibility (**ATTD**) calculations. Fresh fecals were analyzed for fermentative end-products. The BPD (87.0 g/d) caused higher ($P < 0.05$) fecal output (as-is basis) than AMD (62.3 g/d) and CD (58.0 g/d). Fecal score for the BPD (3.1) was greater ($P < 0.05$) than for AMD (2.8) or CD (2.6). Acid-hydrolyzed fat ATTD was lower ($P < 0.05$) for the BPD (94.1%) than for the AMD (95.5%) and CD (95.7%). Crude protein ATTD was greater ($P < 0.05$) for the CD (88.5%) than the AMD (82.2%) or BPD (83.7%). Dogs fed AMD (49.9%) or BPD (51.0%) exhibited greater ($P < 0.05$) TDF ATTD than CD. The fermentative profile for the AMD (233.4, 70.9, 8.8, and 12.0 μmole/g DM, respectively) was similar ($P > 0.05$) to the CD (132.9, 61.7, 7.5, and 9.5 μmole/g DM, respectively) profile, with lower ($P < 0.05$) concentrations of acetate and propionate and higher ($P < 0.05$) concentrations of isovalerate and indoles compared to the BPD. Dogs fed AMD (47.0 μmole/g DM) or BPD (54.2 μmole/g DM) exhibited similar ($P > 0.05$) fecal butyrate concentrations greater ($P < 0.05$) than for CD (24.7 μmole/g DM). Given these

results, avocado meal appears to be an acceptable dietary fiber source when compared to traditional fiber sources used in canine diets.

INTRODUCTION

Avocado meal is an underutilized by-product of the avocado oil processing industry. Even though there is no published research on feeding a diet containing avocado meal to dogs, pet owners are told that avocados are poisonous and should not be fed. This information comes from veterinary case studies, including only one with dogs. Studies testing avocado meal or defatted avocado pulp have found that it is a suitable fiber source and can affect blood cholesterol levels, digestibility of nutrients, and feed efficiency. But all of this work was done with rats (Naveh et al., 2002), sheep (Skenjana et al., 2006), and broiler chickens (van Ryssen et al., 2013) and, therefore, is not directly applicable to dogs. In addition, none of these studies have addressed the potential impact avocado meal may have on gastrointestinal fermentation, which relates to the dietary fiber source used in canine diets and may have implications for gut health.

This study's objective was to compare avocado meal to beet pulp and cellulose, traditional fiber sources in diets for canines, to determine its potential for use in the pet food industry. We hypothesized that avocado meal would not negatively impact digestibility of macronutrients or the fermentation of colonic microbiota.

MATERIALS AND METHODS

Animals

All animal procedures were approved by the University of Illinois Institutional Animal Care and Use committee. Nine intact female dogs [4.9 ± 0.6 yr, 11.98 ± 1.76 kg, and 6.72 ± 1.18 body condition score (**BCS**)] were used in a replicated 3x3 Latin square design. Each period

consisted of 10 d of diet adaptation and 4 d of total fecal and urine collection. Dogs were housed individually (1.2 m x 1.8 m) with free access to water. They were fed twice daily at 0800 and 1600 and had access to food until the next feeding time when food refusals, if present, were collected and recorded. During the collection phase, dogs were housed individually in metabolic cages and given the same access to food and water.

Diets

Three diets were formulated to meet the AAFCO (2016) nutritional requirements for adult cats with similar macronutrient targets and bulk densities. The avocado meal ingredient was obtained from Green Source Organics Natural Extracts (Boynton Beach, FL), all other dry ingredients from Lortscher Animal Nutrition (Bern, KS), and choice white grease from the University of Illinois at Urbana-Champaign Feed Mill (Champaign, IL). The total dietary fiber (**TDF**) source for each diet was either avocado meal (**AMD**), beet pulp (**BPD**), or cellulose (**CD**). Diets were manufactured at the Kansas State University Bioprocessing and Industrial Value Added Products Innovation Center (Manhattan, KS) and coated with an additional 4% choice white grease (University of Illinois Feed Mill, Champaign, IL) and 2% palatant (AFB International bioflavor #F25003, St. Charles, MO). Dogs were fed to maintain BW and body condition score, which were measured once a week during the study periods.

Acceptability testing

Acceptability of the AMD without the second coating of choice white grease and palatant was determined by Kennelwood, Inc. (Champaign, IL) using a one-bowl monadic test. Twenty dogs (13.81 ± 3.90 kg) were offered 400 g/d for 2 d. Daily food intake was calculated by subtracting food refusals from the offered food.

Sample Collection

During the collection phase, all feces were collected from each dog for macronutrient analysis. Fecal weight (as-is) and fecal score using a five point scale (1 = dry, hard pellets; 5 = diarrhea) were recorded for each sample. Samples were frozen at -20 ℃ for analysis at a later time. Total urine output volume after acidifying with 2N hydrochloric acid also was recorded and approximately 25% of each sample was saved for further analysis. Samples from each dog were stored in separate containers and frozen at -20 °C.

One fecal sample from each dog was collected within 15 minutes (**min**) of defecation and analyzed for dry matter (**DM**), phenols and indoles, short-chain fatty acids (**SCFA**), and branched-chain fatty acids (**BCFA**). A pH reading, fecal score, and total sample weight also were taken. Dry matter was measured by drying approximately 2 grams (**g**) of feces in duplicate in a 105 ℃ oven until all moisture was removed. Approximately 2 g of feces in duplicate were stored in plastic tubes covered in parafilm and frozen at -20 ℃ for subsequent indole and phenol analyses. Finally, 5 g of sample were stored in Nalgene bottles containing 5 milliliters (**mL**) of 2N hydrochloric acid and frozen at -20 °C to determine SCFA, BCFA, and ammonia concentrations.

Eight millimeters of blood were collected from each dog on 14 d of each collection period. One milliliter was saved for plasma analysis and the remaining 7 mL were used for serum chemistry. All blood analyses were done by the Clinical Pathology group at the University of Illinois College of Veterinary Medicine (Urbana, IL).

Chemical Analyses

Diets were subsampled and ground in a Wiley mill with a 10 mesh [2 millimeter (**mm**)] screen size. Fresh samples from each dog and period were pooled together and dried in a 57 ℃

oven before grinding in the Wiley Mill with a 10 mesh (2 mm) screen size. These materials were used for macronutrient concentrations. Dry matter, organic matter (**OM**), and ash were determined for the diets and feces using the Association of Official Analytical Chemists 1975 procedure (#942.05). Acid-hydrolyzed fat (**AHF**) in the diet and feces was done following methods of the American Association of Cereal Chemists (Method 30-14), the Official Methods of Analysis of AOAC International (2002), and Budde et al. (1952). Crude protein (**CP**) analysis was done by measuring total nitrogen using a LECO TruMac (model 630-300-300) and following the Official Method of AOAC International (2002). Gross energy of diets, feces, and urine were measured using a Parr 6200 calorimeter (Parr Instrument Company, Moline, IL). Total dietary fiber (**TDF**) was analyzed according to Prosky et al. (1992) and the Official Method of AOAC International, 2006 (Methods 985.29 and 991.43).

Short-chain fatty acids and BCFA were analyzed using gas chromatography with a glass 6'x1/4" ODx4mmID column and 10%SP1200/1%H_3PO_4 on 80/100 Chrom-WAW, Supleco packing and following the methods of Erwin et al. (1961), Supleco Inc. (1975), and Goodall and Byers (1978). Gas chromatography also was used to measure phenols and indoles as cited in Flickinger et al. (2003). Ammonia concentration was determined using the methods of Chaney and Marbach (1962).

Physical Analyses

Ten kibbles of the AMD, BPD, and CD were weighed individually on an analytical scale (model #AG104, Mettler Toledo, Switzerland). Digital calipers (model #01407A, Neiko Tools, China) were used to determine radial diameter and piece length. From these measurements, piece volume, piece density, sectional expansion index (**SEI**), and specific length were calculated

using **Equations 5.1-5.4**. Replicated of each diet were averaged and sample standard deviation was calculated.

Equation 5.1: Piece volume formula

$$Piece\ volume\ (cm^3) = \pi * (Piece\ diameter\ in\ cm)^2 * \frac{(Piece\ length\ in\ cm)}{4}$$

Equation 5.2: Piece density formula

$$Piece\ density\ \left({}^{g}/_{cm^3}\right) = \frac{Piece\ mass\ in\ g}{Piece\ volume\ in\ cm^3}$$

Equation 5.3: Sectional expansion index (SEI) formula

$$SEI = \frac{(Piece\ diameter\ in\ mm)^2}{(Die\ diameter\ in\ mm)^2}$$

Equation 5.4: Specific length formula

$$Specific\ length\ \left({}^{mm}/_{g}\right) = \frac{Piece\ length\ in\ mm}{Piece\ mass\ in\ g}$$

A texture analyzer (TA HD *plus*; Texture Technologies Corporation, Scarsdale, NY) equipped with a 30 kg load cell and a 50.8 mm cylindrical probe (TA-15) was used to compress 10 kibbles per diet by 50%. Pre-test, test, and post-test speed settings were 2, 1, and 10 mm/s, respectively. Data was collected using Texture Expert Exceed software (Stable Micro Systems, Godalming, England). Peak force [greatest force measurement, Newtons (**N**)] and energy required for compression [area under the curve, Newtons x millimeters (**Nxmm**)] were calculated from time vs. force graphs. This method was slightly modified from Dogan et al. (2007). Duplicates for each diet and processing stage combination were averaged together and standard deviation was calculated.

Calculations

Digestibility of individual macronutrients was calculated by subtracting the nutrient content of the feces from the nutrient content of food consumed, dividing by the nutrient content of the food consumed, and multiplying by 100. Nutrient content of feces and diet were determined by multiplying the feces or diet (g, DM basis) by the corresponding nutrient percentage. Digestible energy was calculated by subtracting the energy contained in the feces from the energy contained in the diet. Metabolizable energy was determined by subtracting the energy contained in the urine from the digestible energy.

Statistical Analysis

Data were compared using SAS, version 9.4, using a mixed model. The statistical model included the fixed effect of diet and the random effect of animal. Data normality was checked using the UNIVARIATE procedure of SAS. All treatment least-square means were compared with each other and Tukey adjustment was used to control for experiment-wise error. P-values less than 0.05 were considered statistically different, whereas p-values less than 0.10 but greater than 0.05 were considered to represent a trend.

RESULTS

Ingredients and their inclusion levels were similar among dietary treatments (**Table 5.1**). Avocado meal and beet pulp replaced cellulose and chicken by-product meal and brewer's rice. Choice-white grease inclusions were adjusted to maintain isonitrogenous and isocaloric nutrient compositions (**Table 5.2**). Small differences in AHF were observed, with CD (18.5%, DM basis) being the greatest, followed by AMD (17.8%, DM basis) and BPD (17.0%, DM basis). The treatments also had different IDF:SDF ratios.

Physical characteristics of the kibbles varied across dietary treatments (**Table 5.3**). Piece mass (AMD = 0.14 ± 0.01 g; BPD = 0.15 ± 0.01 g; CD = 0.12 ± 0.01 g) and piece length (AMD = 6.6 ± 0.6 mm; BPD = 6.7 ± 0.5 mm; CD = 6.7 ± 0.4 mm) were very similar. The AMD (7.8 ± 0.4 mm) had the largest piece diameter with BPD (6.8 ± 0.3 mm) and CD (6.5 ± 0.3 mm) being smaller. The same relationship was observed for piece volume (AMD = 0.32 ± 0.04 cm^3; BPD = 0.25 ± 0.03 cm^3; CD = 0.23 ± 0.03 cm^3) and SEI (AMD = 3.8 ± 0.4; BPD = 2.9 ± 0.3; CD = 2.7 ± 0.3). The BPD (0.61 ± 0.05 g/cm^3) had greater piece density, followed by CD (0.55 ± 0.07 g/cm^3) and AMD (0.46 ± 0.05 g/cm^3). On the other hand, AMD (4.6 ± 0.2 mm/g) and BPD (4.5 ± 0.1 mm/g) had similar specific length and were smaller than CD (5.5 ± 0.4 mm/g). The BPD had the lowest kibble hardness (32.4 ± 5.0 N) and energy to compress (24.8 ± 7.5 Nxmm), with AMD being very similar (48.4 ± 10.2 N and 35.9 ± 10.3 Nxmm, respectively) and CD being much higher than both (88.2 ± 7.7 N and 110.3 ± 18.3 Nxmm, respectively).

Daily DM intake exhibited a trend ($P < 0.10$), with cats consuming AMD (134.4 g/d) having the lowest intake, those consuming BPD (155.7 g/d) the greatest intake, and the CD treatment (143.0 g/d) being intermediate and not different ($P > 0.10$) than the other two (**Table 5.4**). There was no statistical difference ($P > 0.05$) in intakes of OM, AHF, CP, and gross energy for all dietary treatments. The BPD (87.0 g/d as-is) resulted in greater ($P < 0.05$) daily fecal output on an as-is basis than the CD (58.0 g/d as-is) and the AMD (62.3 g/d as-is), but statistical differences disappeared ($P > 0.05$) when expressed on a DM basis (AMD = 27.6 g/d DM basis; BPD = 30.0 g/d DM basis; CD = 32.2 g/d DM basis). Average fecal score for dogs consuming BPD (3.1) was greater ($P < 0.05$) than for the CD (2.6) and the AMD (2.8). The CD (40.2%) also had the lowest ($P < 0.05$) fecal DM content, followed by AMD (35.3%; $P < 0.05$), then BPD (30.4%; $P < 0.05$). Fecal pH for the CD (6.2) was higher ($P < 0.05$) than for the AMD

(5.9) and BPD (5.5), which were not different from each other (*P > 0.05*). Apparent total tract digestibility (ATTD) of DM, OM, and GE were similar among dietary treatments (*P > 0.05*). Dogs consuming BPD (94.1% DM basis) exhibited lower (*P < 0.05*) AHF ATTD than those consuming CD (95.7% DM basis) or AMD (95.5% DM basis), which were not different from each other (*P > 0.05*). For CP ATTD, CD (88.5% DM basis) was higher (*P < 0.05*) than AMD (82.2% DM basis) and BPD (83.7% DM basis), which did not differ from each other (*P > 0.05*). Energy partitioning into digestible energy and metabolizable energy did not differ (*P > 0.05*) by dietary treatment.

Consuming the BPD (672.7 μmole/g DM and 480.5 μmole/g DM, respectively) resulted in the greatest (*P < 0.05*) total SCFA and acetate concentrations, with AMD (351.0 μmole/g DM and 233.4 μmole/g DM, respectively) and CD (219.0 μmole/g DM and 132.9 μmole/g DM, respectively) not different from each other (*P > 0.05*) (**Table 5.5**). The concentration of butyrate was lowest (*P < 0.05*) for dogs consuming CD (24.7 μmole/g DM), and AMD (47.0 μmole/g DM) and BPD (54.2 μmole/g DM) were the same (*P > 0.05*). The BPD (138.0 μmole/g DM) resulted in the greatest (*P < 0.05*) propionate concentration, while AMD (70.9 μmole/g DM) and CD (61.7 μmole/g DM) were lower and not statistically different from each other (*P > 0.05*). Total BCFA concentration was not impacted (*P > 0.05*) by dietary treatment, but individual compounds were. Isobutyrate concentration tended (*P < 0.10*) to be greater for dogs fed AMD (5.0 μmole/g DM) and CD (5.0 μmole/g DM) in contrast to those fed BPD (3.3 μmole/g DM). Statistical differences were observed in the concentration of isovalerate, with AMD- (8.8 μmole/g DM) and CD-fed dogs (7.5 μmole/g DM) being similar (*P > 0.05*) to each other, but greater (*P < 0.05*) than BPD-fed dogs (5.6 μmole/g DM). Valerate concentration for dogs consuming BPD (1.0 μmole/g DM) was greater (*P < 0.05*) than CD (0.5 μmole/g DM), and

AMD (0.8 μmole/g DM) was not different ($P > 0.05$) from either. Concentrations of fecal indole and total indole and phenol exhibited the same relationship, with AMD (12.0 μmole/g DM and 12.1 μmole/g DM, respectively) and CD (9.5 μmole/g DM and 9.9 μmole/g DM, respectively) being similar ($P > 0.05$) but higher ($P < 0.05$) than BPD (2.9 μmole/g DM and 2.9 μmole/g DM, respectively). Ammonia concentrations for CD (132.9 μmole/g DM) were higher ($P < 0.05$) than for the BPD (90.7 μmole/g DM), with AMD (109.3 μmole/g DM) being intermediate ($P > 0.05$).

Many serum metabolites were not impacted ($P > 0.05$) by the dietary treatments and few were outside the acceptable range for healthy adult dogs (**Table 5.6**). Albumin levels were within reference ranges, but were greatest ($P < 0.05$) for dogs fed AMD (3.3 mg/dL), lowest for those fed CD (3.2 mg/dL; $P < 0.05$), and the BPD (3.2 mg/dL) was not different ($P > 0.05$) from either. The BPD (2.6 mg/dL) exhibited globulin levels below the reference range (add value) when fed to dogs, but there was no treatment effect ($P > 0.05$). The ratio of albumin to globulin was also not affected ($P > 0.05$), but all treatments (AMD = 1.2; BPD = 1.2; CD = 1.2) exhibited a ratio higher than the reference range (add value). Serum calcium levels were within reference ranges, but were greater ($P < 0.05$) for dogs fed AMD (10.3 mg/dL) than CD (10.1 mg/dL), but BPD (10.2 mg/dL) was not different ($P > 0.05$) from either. Potassium levels exhibited a trend for CD (4.5 mmol/L) to be greater ($P < 0.10$) than AMD (4.3 mmol/L) when fed to dogs, but BPD (4.4 mmol/L) was not different ($P > 0.10$) from either. The reverse was seen in the ratio of sodium to potassium, with AMD (34.1) being greater ($P < 0.10$) than CD (32.4), and BPD (32.7) not differing from either ($P > 0.10$). The BPD (26.5 U/L) and CD (25.2 U/L) resulted in levels of alkaline phosphorus total that were similar ($P > 0.05$), but lower ($P < 0.05$) than the AMD (51.7 U/L). The same relationship was observed for corticosteroid-induced alkaline phosphatase: AMD (22.6) was greater ($P < 0.05$) than BPD (8.9) and CD (6.3), which were not different ($P >$

0.05) from each other. The BPD (0.2 mg/dL) resulted in greater (*P < 0.05*) levels of total bilirubin than CD (0.1 mg/dL), and AMD (0.2 mg/dL) was intermediate and not different (*P > 0.05*) from either. Cholesterol levels were the highest (*P < 0.05*) for dogs fed the AMD (236.0 mg/dL) and BPD (198.0 mg/dL) and CD (209.2 mg/dL) were not different (*P > 0.05*) from each other. Dogs fed the CD (84.1 mg/dL) had a greater (*P < 0.05*) level of triglycerides compared to those fed the AMD (53.6 mg/dL), with BPD (64.7 mg/dL) not being different (*P > 0.05*) from either. The reverse relationship was observed with bicarbonate levels: AMD (21.2 mmol/L) was higher (*P < 0.05*) than CD (19.6 mmol/L), and BPD (20.4 mmol/L) was intermediate and not different (*P > 0.05*) from AMD and CD.

The complete blood counts were within normal references ranges for adult canines, except for hematocrit (AMD = 55.5%; BPD = 55.4%; CD = 55.2), which was above the reference range for all treatments but not different (*P > 0.05*) from each other (**Table 5.7)**. Blood lymphocytes trended to be higher (*P < 0.10*) for BPD (1.7 x 10^3/ μL) than the CD (1.3 x 10^3/ μL), with AMD (1.4 x 10^3/ μL) being intermediate (*P > 0.10*). All other cell counts were not affected (*P > 0.05*) by the dietary treatments.

Monadic testing resulted in an average daily food intake of 172.1 ± 68.2 g for d 1, 169.8 ± 55.1 g for d 2, and 341.9 ± 119.3 g for both d (**Figure 5.1**).

DISCUSSION

Fiber in canine diets has become a greater concern with recent research suggested that fiber aid with weight loss and supporting beneficial bacteria in the gastrointestinal tract. Avocado meal has not been evaluated as a fiber source for canines, nor has the impact of high fiber formulations on physical and organoleptic characteristics of extruded foods been described. As

such, the aim of this study was to: 1) describe kibbles with high concentrations of different fiber sources in terms of physicality and texture, and 2) compare AMD to BPD and CD in terms of macronutrient ATTD and concentrations of fermentative end-products.

In general, the physical and organoleptic results from this study agree with previously published findings from research on human food products. Kibble hardness and energy to compress for AMD and BPD were lower than for CD. This was driven by the differences in SDF and IDF, and was previously observed by Yanniotis et al. (2007) who found that extrudates higher in IDF were less expanded than those higher in SDF. Our results do not support that, but our main processing goal was to produce dietary treatments with similar bulk density, piece diameter, and piece length. Different processing settings have the ability to change the degree of expansion (Lue et al., 1991; Rinaldi et al., 2000) and it might confound our results.

Standard fiber sources (BPD and CD) showed results similar to previously published literature. Even though our BPD was high in TDF (> 15%, DM basis), our ATTD from our BPD diet are well-aligned with those reported by Fahey et al. (1990), who tested the effects of 6 levels of beet pulp (0.0%, 2.5%, 5.0%, 7.5%, 10.0%, and 12.5%) in diets for canines and found 7.5%, DM basis, of beet pulp to be ideal. In addition, and agreeing with Fahey et al. (1990), we observed an increased fecal output on as-is basis for dogs fed the BPD that was not observed for dogs fed the AMD or CD. This is likely due to the water holding capacity of beet pulp fiber. Howard et al. (2000) also reported no differences ($P > 0.10$) in DM digestibility and DM intake between cellulose (6% DM basis) and beet pulp (6% DM basis), which was also observed in the present study, when they compared them in a study with diets containing a fiber blend (6.0% beet pulp, 2.0% gum talha, and 1.5% fructooligosaccharides, DM basis) or fructooligosaccharides (1.5% DM basis) and a no-fiber diet. Our study results agree with their

finding that beet pulp and cellulose do not result in different GE or nitrogen/CP intakes. The finding that there are no differences in nitrogen/CP digestibility between the two sources was not found in the present study. Howard et al. (2000) found that beet pulp was more fermentable than cellulose, meaning that more nutrients were available to the gastrointestinal microbiota when the dogs were fed beet pulp. This would result in greater concentrations of fermentative end-products for BPD, which was seen in total SCFA, acetate, butyrate, propionate, and valerate. Other fermentation end products were either not significant or greater for CD, but overall BPD and CD performed as expected.

Although previous research on avocado meal and defatted avocado pulp is limited, some of it agrees with the results obtained with the AMD. Naveh et al. (2002) also observed decreased fecal output (as-is) for defatted avocado pulp compared to cellulose when fed to rats. They also found that as-is food intake for defatted avocado pulp was lower than for cellulose; this was observed in the numerically, but not significantly, lower DM intake for AMD compared to CD. We may have observed stronger significance if expressed on an as-is basis. Our conclusion that avocado meal may be a good fiber source for canines supports the work of van Ryssen et al. (2013), who tested multiple levels of avocado meal in complete diets for broiler chickens and found that increased levels of avocado meal decreased production, as is expected with higher levels of fiber.

Avocado meal performed similarly to cellulose in canine diets. Fermentability of the fiber sources likely played a role in the results. Bosch et al. (2009) found differences when feeding a highly fermentable canine diet (8.5% as-is beet pulp, 2% as-is inulin) versus a poorly fermentable canine diet (8.5% as-is cellulose). In their study, food intake tended to be lower for dogs fed the highly fermentable diet. In our study, AMD-fed dogs trended to have lower

voluntary DM intake than BPD, but CD was not different from either. Bosch et al. (2009) also reported decreased ATTD of DM, OM, neutral detergent fiber, acid detergent fiber, non-starch polysaccharides, and energy and increased ATTD of crude fat when the high fermentability diet was consumed. The only result confirmed in our study is the greater AHF ATTD observed in AMD and CD compared to BPD. Dry matter, OM, and energy ATTD were not significantly impacted in our study. However, CP ATTD for AMD and BPD was reduced when compared to CD. A potential reason is higher excretion of microbial protein in the feces of dogs fed AMD and BPD due to greater gut fermentation.

Bosch et al. (2009) also measured short-chain fatty acids and found increased concentrations of total SCFA, acetate, and propionate when dogs were fed the highly fermentable diet vs. the poorly fermentable diet. A highly fermentable diet will provide a greater pool of substrate for gut microbes to ferment, resulting in greater concentrations of fermentative end-products. Our study showed lower total SCFAs, acetate, butyrate, and propionate concentrations for CD than BPD, which was expected because beet pulp is more fermentable than cellulose. Unexpectedly, fecal concentrations of total SCFAs, acetate, and propionate from dogs fed AMD were not different from dogs fed CD and concentration of butyrate was not different from dogs fed BPD. This, in conjunction with the results from Bosch et al. (2009), further suggests that avocado meal fermentability is intermediate between beet pulp and cellulose.

Overall, the dogs in this study remained healthy on all treatments. No serum metabolites were outside acceptable ranges. Hematocrit was above the range for healthy canines for all treatments, but only by 3%. This is not great enough to cause concern. Urine of AMD-fed and

BPD-fed dogs appeared darker than CD-fed dog urine. This could be caused by the presence of anthocyanins in beet pulp and avocado meal, mainly coming from the skin (Ashton et al., 2006).

No clinical signs of persin toxicity were exhibited in this study. Published case studies have listed serum chemistry results observed while consuming portions of avocado. The Grant et al. 1991 study in rats showed elevated blood urea nitrogen, whereas the dogs in our study had normal levels and did not exhibit treatment effects. Moderate kidney disease was observed in a hen fed 12.5 g of avocado leaves per kg of BW and a hen fed 15 g of unripe fruit per kg of BW (Burger et al., 1994). Consumption of AMD in this study resulted in normal serum creatinine levels comparable to BPD and CD. A case study in two dogs suspected of suffering from avocado toxicity reported normal blood urea nitrogen and protein, but elevated white blood cells and serum alanine aminotransferase and alkaline phosphatase levels (Buoro et al., 1994). Dogs in this study had statistically higher alkaline phosphorus total when fed AMD compared to BPD and CD, but these levels were within normal ranges. There was also no observed effect on alanine amino transferase or white blood cells, which does not agree with Buoro et al. (1994). Blood serum in rabbits fed avocado leaves had elevated sodium levels and lower chloride and phosphorus levels than acceptable (Ali et al., 2010). The present study showed no treatment effects on sodium and phosphorus levels, and all levels were within normal ranges. Chloride levels were significantly lower for AMD compared to BPD and CD, but the levels were still acceptable and the differences too small to be detected clinically. The only blood count outside of acceptable ranges was hematocrit, which was only slightly elevated and not mentioned in any reported case of persin toxicity. Avocado meal-fed dogs did not exhibit abnormal levels of lymphocytes and neutrophils, as was seen in Buoro et al. (1994). These results make it highly unlikely that persin toxicity is a concern when feeding a diet containing avocado meal. Total

cholesterol was also significantly higher in AMD, but was still within reference ranges. This treatment effect was also seen in rats (Naveh et al., 2002; Imafidon et al., 2010); the researchers believe this occurred because the supplemented levels of avocado pulp or aqueous avocado seed extract, respectively, were above the ideal supplementation. A commercial diet would not contain as much avocado meal as this experimental diet (18.67%), which means that any of the serum chemistry results from this study may be diminished with a commercial formulation.

One concern with fiber sources is potentially negative impacts on palatability (Fekete et al., 2001). The monadic test revealed that acceptability of AMD was adequate (approx. 150 g/d) and similar to the daily food intake results observed during the digestibility study (approx. 143 g/d). Even though the monadic test and digestibility trial were not done using the same dogs, both trials used Beagle dogs of similar BW. Because the dogs in the monadic test did not overeat the AMD diet, the addition of extra topical fat and palatant can improve food palatability and ensure appropriate food intake.

CONCLUSION

Avocado meal appears to be an acceptable dietary fiber source for adult canines. The present study shows that avocado meal performs more similarly to cellulose in a high TDF diet in terms of ATTD and fecal fermentative end-product concentrations. All differences in blood analyses were within acceptable ranges, which suggests that dogs can maintain good health while on this diet. In addition, a commercially produced diet likely will not contain this much avocado meal, so the negative impacts on food palatability could be diminished. A diet formulated with less avocado meal also may not need as much choice white grease and palatant. Future work

should develop and evaluate fiber blends incorporating avocado meal to examine their potential functionality for gut and animal health.

TABLES AND FIGURE

Table 5.1: Ingredient composition of dietary treatments with select dietary fiber sources for adult canines.

	Treatments[1]		
Ingredient, % as-is	AMD	BPD	CD
Chicken by-product meal	29.57	29.91	31.42
Brewer's rice	26.81	27.17	31.61
Choice white grease	9.11	11.32	11.32
Corn gluten meal	7.66	7.74	7.74
Whole corn	3.83	3.87	3.87
AFB bioflavor F25003	1.89	1.89	1.89
Avocado meal	18.67	0.00	0.00
Beet pulp	0.00	15.66	0.00
Cellulose	0.00	0.00	9.72
AFB bioflavor B22006	0.89	0.89	0.89
Salt	0.48	0.47	0.47
Potassium chloride	0.43	0.42	0.42
Taurine	0.19	0.19	0.19
Mineral premix	0.17	0.17	0.17
Vitamin premix	0.17	0.17	0.17
Choline chloride	0.12	0.12	0.12

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

[2] Provided per kilogram of diet: 35.6 mg manganese ($MnSO_4$), 601.4 mg iron ($FeSO_4$), 29.6 mg copper ($CuSO_4$), 0.02 mg cobalt ($CoSO_4$), 326.3 mg zinc ($ZnSO_4$), 2.9 mg iodine (KI), and 0.8 mg selenium (Na_2SeO_3).

[3] Provided per kilogram of diet: 17000 IU vitamin A (retinyl acetate), 2550 IU vitamin D3, 136 IU vitamin E (DL-α tocopherol acetate), 3.3 mg vitamin K, 28.9 mg thiamin, 28.9 mg riboflavin, 51.7 mg pantothenic acid, 117.3 mg niacin, 28.9 mg pyridoxine, 0.1 mg biotin, 1.0 mg folic acid, and 1.1 mg vitamin B12 (mannitol).

Table 5.2: Chemical composition of dietary treatments containing select fiber sources for adult canines.

	Treatments[1]		
Item	AMD	BPD	CD
Dry matter, %	95.3	93.4	95.2
		----- % DM basis -----	
Organic matter	91.3	91.8	92.8
Ash	8.7	8.2	7.2
Acid hydrolyzed fat	17.8	17.0	18.5
Crude protein	32.5	32.3	32.4
Total dietary fiber	17.7	18.1	19.5
Soluble dietary fiber	4.3	3.5	2.6
Insoluble dietary fiber	13.4	14.7	17.0
Gross energy, kcal/g	5.3	5.2	5.3

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

Table 5.3: Analyzed physical characteristics of extruded dietary treatments for adult canines containing select dietary fiber sources.

	Treatments[1] (Average ± Standard Deviation)		
Item	AMD	BPD	CD
Piece mass, g	0.14 ± 0.01	0.15 ± 0.01	0.12 ± 0.01
Piece diameter, mm	7.8 ± 0.4	6.8 ± 0.3	6.5 ± 0.3
Piece length, mm	6.6 ± 0.6	6.7 ± 0.5	6.7 ± 0.4
Piece volume, cm^3	0.32 ± 0.04	0.25 ± 0.03	0.23 ± 0.03
Piece density, g/cm^3	0.46 ± 0.05	0.61 ± 0.05	0.55 ± 0.07
Sectional expansion ratio	3.8 ± 0.4	2.9 ± 0.3	2.7 ± 0.3
Specific length, mm/g	4.6 ± 0.2	4.5 ± 0.1	5.5 ± 0.4
Kibble hardness, N	48.4 ± 10.2	32.4 ± 5.0	88.2 ± 7.7
Energy to compress 50%, Nxmm	35.9 ± 10.3	24.8 ± 7.5	110.3 ± 18.3

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

Table 5.4: Food intake, fecal characteristics, and total tract apparent macronutrient digestibility by adult canines fed dietary treatments containing select dietary fiber sources.

	Treatments[1]			
Item	AMD	BPD	CD	SEM[†]
Food intake, as-is				
Dry matter, g/d	134.4[y]	155.7[x]	143.0[xy]	13.61
Organic matter, g/d	135.9	142.9	141.1	7.57
Acid hydrolyzed fat, g/d	26.5	26.5	28.2	1.44
Crude protein, g/d	48.4	50.4	49.3	2.67
Total dietary fiber, g/d	27.3[y]	28.3[xy]	29.6[x]	1.40
Soluble dietary fiber, g/d	6.6[a]	5.4[b]	3.8[c]	0.26
Insoluble dietary fiber, g/d	20.7[b]	22.8[b]	25.8[a]	1.14
Gross energy, kcal/d	781.6	810.5	805.9	43.15
Fecal output, g/d (as is)	62.3[b]	87.0[a]	58.0[b]	8.30
Fecal output, g/d (DMB)	27.6	30.0	32.2	2.11
Fecal score	2.8[b]	3.1[a]	2.6[b]	0.10
Fecal DM %	35.3[b]	30.4[c]	40.2[a]	1.39
Fecal pH	5.9[b]	5.5[b]	6.2[a]	0.10
Digestibility, %				
Dry matter	81.7	80.8	79.5	1.75
		-----% DM basis -----		
Organic matter	84.5	84.3	81.9	0.94
Acid hydrolyzed fat	95.5[a]	94.1[b]	95.7[a]	0.39
Crude protein	82.2[b]	83.7[b]	88.5[a]	1.29
Total dietary fiber	49.9[a]	51.0[a]	33.5[b]	3.01
Energy	85.3	85.6	80.0	0.85
Digestible energy, kcal/g	4.5	4.5	4.5	0.04
Metabolizable energy, kcal/g	4.2	4.2	4.2	0.04

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

[a-c] Superscripts with different letters in a row represent statistical differences ($P < 0.05$).

[x-y] Superscripts with different letters in a row represent trending differences ($0.05 < P < 0.10$).

[†] Pooled standard error of means.

Table 5.5: Fecal fermentative-end products for adult canines fed dietary treatments with select dietary fiber sources.

	Treatments[1]			
Item, μmole/g DM	AMD	BPD	CD	SEM[†]
Total short-chain fatty acids	351.0[b]	672.7[a]	219.0[b]	40.55
Acetate	233.4[b]	480.5[a]	132.9[b]	32.21
Butyrate	47.0[a]	54.2[a]	24.7[b]	3.28
Propionate	70.9[b]	138.0[a]	61.7[b]	8.09
Total branched-chain fatty acids	14.7	9.9	14.2	2.14
Isobutyrate	5.0[x]	3.3[y]	5.0[x]	0.41
Isovalerate	8.8[a]	5.6[b]	7.5[a]	0.90
Valerate	0.8[ab]	1.0[a]	0.5[b]	0.09
Ammonia	109.3[ab]	90.7[b]	132.9[a]	10.60
Total indoles and phenols	12.1[a]	2.9[b]	9.9[a]	1.37
Indole	12.0[a]	2.9[b]	9.5[a]	1.33
Phenol	0.2	0.1	0.4	0.12

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.
[a-b] Superscripts with different letters in a row represent statistical differences ($P < 0.05$).
[x-y] Superscripts with different letters in a row represent trending difference ($0.05 < P < 0.10$).
[†] Pooled standard error of means.

Table 5.6: Fasted serum chemistry profiles for adult canines fed dietary treatments with select dietary fiber sources.

Item	Reference Range	Treatments[1]			SEM[†]
		AMD	BPD	CD	
Creatinine, mg/dL	0.5 - 1.5	0.5	0.5	0.5	0.02
Blood urea nitrogen, mg/dL	6 - 30	12.1	12.7	13.1	0.4703
Total protein, g/dL	5.1 - 7.0	6.0	5.9	5.9	0.09
Albumin, g/dL	2.5 - 3.8	3.3^{a}	3.2^{ab}	3.2^{b}	0.05
Globulin, g/dL	2.7 - 4.4	2.7	2.6	2.7	0.08
Albumin/globulin ratio	0.6 - 1.1	1.2	1.2	1.2	0.04
Calcium, mg/dL	7.6 - 11.4	10.3^{a}	10.2^{ab}	10.1^{b}	0.10
Phosphorus, mg/dL	2.7 - 5.2	3.4	3.7	3.7	0.17
Sodium, mmol/L	141 - 152	144.7	144.7	144.4	0.42
Potassium, mmol/L	3.9 - 5.5	4.3^{y}	4.4^{xy}	4.5^{x}	0.09
Sodium/potassium ratio	28 - 36	34.1^{x}	32.7^{xy}	32.4^{y}	0.74
Chloride, mmol/L	107 - 118	109.8^{b}	111.2^{a}	111.9^{a}	0.62
Glucose, mg/dL	68 - 126	90.7	88.2	91.2	2.73
Alkaline phosphatase total, U/L	7 - 92	51.7^{a}	26.5^{b}	25.2^{b}	4.59
Corticosteroid-induced alkaline phosphatase, U/L	0 - 40	22.6^{a}	8.9^{b}	6.3^{b}	3.12
Alanine aminotransferase, U/L	8 - 65	34.0	34.1	32.3	4.06
Gamma-glutamyl transferase, U/L	0 - 7	3.6	3.8	3.6	0.17
Total bilirubin, mg/dL	0.1 - 0.3	0.2^{ab}	0.2^{a}	0.1^{b}	0.02
Cholesterol total, mg/dL	129 - 297	236.0^{a}	198.0^{b}	209.2^{b}	14.73
Triglycerides, mg/dL	35 - 154	53.6^{b}	64.7^{ab}	84.1^{a}	10.02
Bicarbonate (TCO_2), mmol/L	16 - 24	21.2^{a}	20.4^{ab}	19.6^{b}	0.53
Anion gap	8 - 25	17.9	17.6	17.7	0.68

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet.

[a-b] Superscripts with different letters in a row represent statistical differences ($P < 0.05$).

[x-y] Superscripts with different letters in a row represent trending difference ($0.05 < P < 0.10$).

[†] Pooled standard error of means.

Table 5.7: Fasted complete blood cell analysis for adult canines fed dietary treatments with select dietary fiber sources.

		Treatments[1]			
Item	Reference range	AMD	BPD	CD	SEM[†]
Red blood cells, $x10^6/\mu L$	5.50-8.50	7.4	7.4	7.4	0.22
Hemoglobin, g/dL	12.0-18.0	17.8	17.7	17.7	0.47
Hematocrit, %	35.0-52.0	55.5	55.4	55.2	1.47
Mean cell volume, fl	60.0-77.0	74.6	74.8	75.1	0.57
Mean corpuscular hemoglobin, pg	20.0-25.0	24.0	24.0	24.1	0.21
Mean corpuscular hemoglobin concentration, g/dL	32.0-36.0	32.1	32.0	31.2	0.11
Platelet estimate, $x10^3/\mu L$	200-900	312.2	316.2	321.7	30.68
White blood cell count, $x10^3/\mu L$	6.00-17.00	6.6	7.1	7.2	0.37
Sequential neutrophils, $x10^3/\mu L$	3.00-11.50	4.7	5.0	5.2	0.36
Band neutrophils, $x10^3/\mu L$	0.00-0.30	0.0	0.0	0.0	0.03
Lymphocytes, $x10^3/\mu L$	1.00-4.80	1.4^{xy}	1.7^{x}	1.3^{y}	0.19
Monocytes, $x10^3/\mu L$	0.20-1.40	0.3	0.3	0.4	0.07
Eosinophils, $x10^3/\mu L$	0.10-1.00	0.2	0.2	0.2	0.05
Basophils, $x10^3/\mu L$	0.00-2.00	0.0	0.0	0.0	0.00

[1] AMD = avocado meal diet; BPD = beet pulp diet; CD = cellulose diet

[x-y] Superscripts with different letters in a row represent trending differences (*0.05 < P < 0.10*).

[†] Pooled standard error of means.

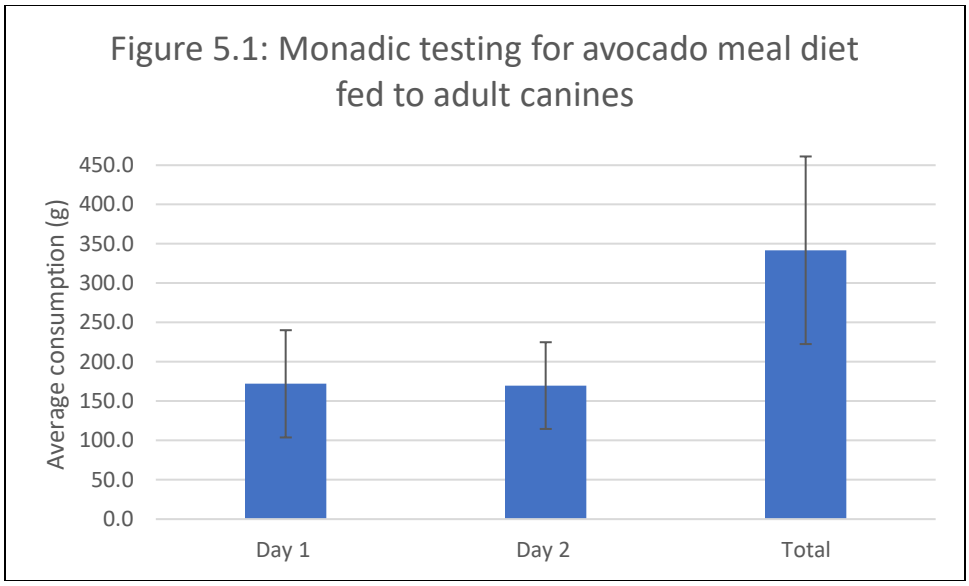
Figure 5.1: Monadic testing for avocado meal diet fed to adult canines
Average consumption (g)
450.0
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Day 1
Day 2
Total

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CHAPTER 6

CONCLUSION

The work detailed in this thesis has many important implications for the pet food industry. First, the relationship between processing extruded pet foods and their ingredient formulations is complicated. We saw that processing settings must be altered to produce extrudates that appear similar to the naked eye, and even with those changes, the products may not yield similar physical and organoleptic measurements. The biggest driver of these differences, especially in texture analysis, was the fractions of soluble dietary fiber and insoluble dietary fiber.

Second, avocado meal is tolerated well by both cats and dogs. In cats, avocado meal performed most similarly to beet pulp in terms of apparent total tract macronutrient digestibility and in fecal fermentation end-products produced. Avocado meal, when fed to dogs, acted most similarly to cellulose, especially in fecal fermentation end-products, but numerically was intermediate between beet pulp and cellulose. Both species exhibited acceptable fecal scores and no negative impacts on fecal output were observed. The experimental diets were not accepted well, but were better after additional coating of choice white grease and palatant. This suggests that such a high inclusion of dietary fibers may not be appropriate for a diet long-term.

Third, avocado meal does not appear to be toxic when included in a pet food formulation. All animals in our studies remained healthy while consuming the diets, and abnormal bloodwork as described in published avocado toxicity case studies with other species were not observed. While there were some treatment differences, these findings might not be replicated in a commercial diet containing less avocado meal.

Moving forward, avocado meal is an acceptable dietary fiber source for the pet food industry. With attention to the required changes in processing, pet food companies should look to this ingredient as a new option for meeting the nutritional needs of cats and dogs while responsibly producing sustainable foods for the growing pet population.